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Diffusion and sorption of organic micropollutants in biofilms with varying thicknesses

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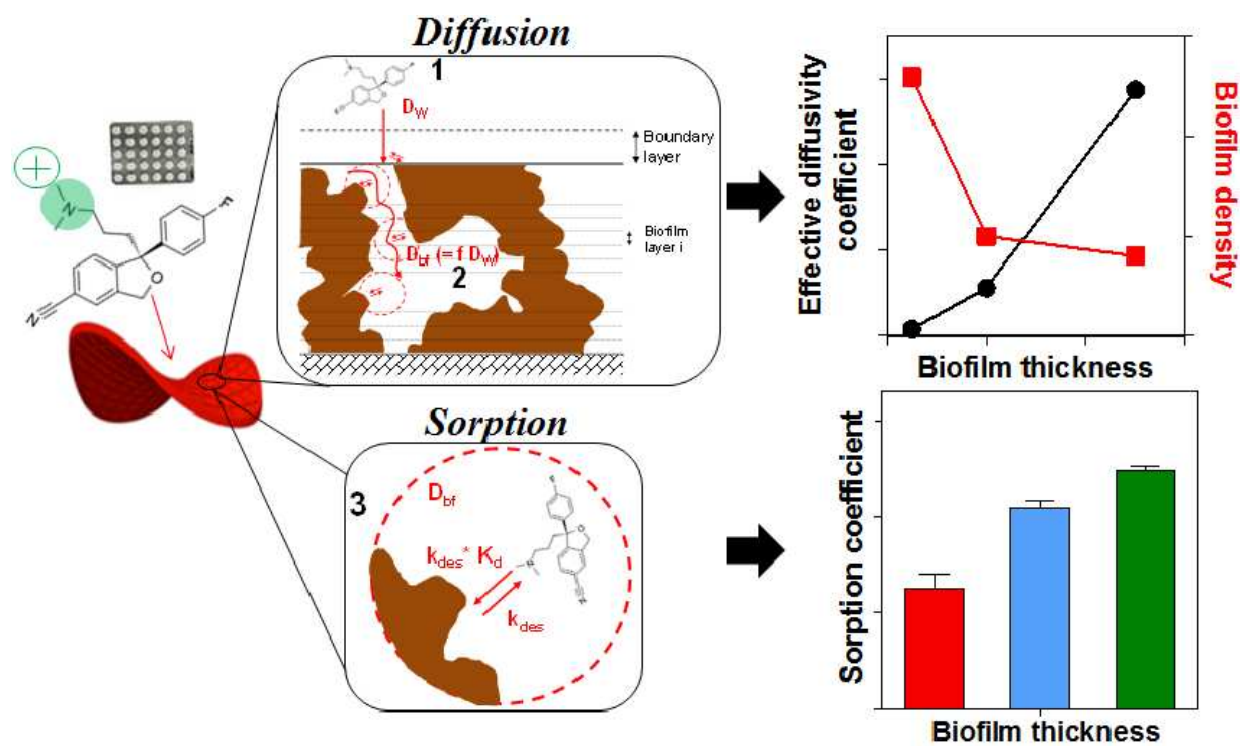
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Abstract

Solid-liquid partitioning is one of the main fate processes determining the removal of micropollutants in wastewater. Little is known on the sorption of micropollutants in biofilms, where molecular diffusion may significantly influence partitioning kinetics. In this study, the diffusion and the sorption of 23 micropollutants were investigated in novel moving bed biofilm reactor (MBBR) carriers with controlled biofilm thickness (50, 200 and 500 μm) using targeted batch experiments (initial concentration = $1 \mu\text{g L}^{-1}$, for X-ray contrast media $15 \mu\text{g L}^{-1}$) and mathematical modelling. We assessed the influence of biofilm thickness and density on the dimensionless effective diffusivity coefficient f (-, equal to the biofilm-to-aqueous diffusivity ratio) and the distribution coefficient $K_{d,eq}$ (L g^{-1}). Sorption was significant only for eight positively charged micropollutants (atenolol, metoprolol, propranolol, citalopram, venlafaxine, erythromycin, clarithromycin and roxithromycin), revealing the importance of electrostatic interactions with solids. Sorption equilibria were likely not reached within the duration of batch experiments (4 h), particularly for the thickest biofilm, requiring the calculation of the distribution coefficient $K_{d,eq}$ based on the approximation of the asymptotic equilibrium concentration ($t > 4 \text{ h}$). $K_{d,eq}$ values increased with increasing biofilm thickness for all sorptive micropollutants (except atenolol), possibly due to higher porosity and accessible surface area in the thickest biofilm. Positive correlations between $K_{d,eq}$ and micropollutant properties (polarity and molecular size descriptors) were identified but not for all biofilm thicknesses, thus confirming the challenge of improving predictive sorption models for positively charged compounds. A diffusion-sorption model was developed and calibrated against experimental data, and estimated f values also increased with increasing biofilm thickness. This indicates that diffusion in thinner biofilms may be strongly limited ($f \ll 0.1$) by the higher biomass density (lower porosity) compared to thicker biofilms.

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48 **Keywords:** Pharmaceuticals, wastewater, moving bed biofilm reactor, partitioning, biofilm

49 density, ionizable chemicals

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1. Introduction

In wastewater treatment systems, partitioning of organic micropollutants to solid matrices is one of the mechanisms leading to their removal from the aqueous phase. The extent of partitioning is typically compound-dependent, and is governed by its affinity for organic phase (i.e., hydrophobic partitioning) and/or by electrostatic and other similar interactions between ionized molecules and charged solid surfaces (i.e., non-hydrophobic partitioning) (Franco and Trapp, 2008; Hyland et al., 2012; Ternes et al., 2004; Mackay and Vasudevan, 2012; Polesel et al., 2015).

Partitioning describes the distribution of molecules between the aqueous and the solid phase. At equilibrium, sorption and desorption rates are equal, and the ratio of sorbed and dissolved concentrations—normalized to the concentration of solids—is defined as the (linear) solid-liquid partition coefficient K_d (expressed in units of L kg^{-1} or, alternatively, L g^{-1}) (Joss et al., 2006; Ternes et al., 2004). Non-linear expressions (Freundlich and Langmuir isotherms) have been also used to describe partitioning equilibria to account for saturation of solid surfaces or synergistic effects (Delle Site, 2001).

Solid-liquid partitioning has been characterized for activated sludge biomass for a high number of pharmaceuticals. Considerably less evidence is available for wastewater treatment biofilms, being limited to antibiotics (sulfamethoxazole, erythromycin, ciprofloxacin, tetracycline) and psycho-active drugs (fluoxetine) in biofilters (Wunder et al., 2011) and granules (Alvarino et al., 2015; Shi et al., 2011). Additionally, partitioning kinetics of other organic contaminants (polycyclic aromatic hydrocarbons, estrogens, nonylphenols, biocides) have been assessed for pure culture biofilms (Wicke et al., 2008, 2007) and river biofilms (Headley et al., 1998; Writer et al., 2011).

Although considered a fast process, partitioning is influenced by mass transfer limitation through diffusive boundary layers and inside the solid matrices, which likely determines the

time needed to achieve equilibrium between aqueous and sorbed concentrations (Joss et al., 2004, 2006). While for activated sludge the equilibrium time is sufficiently fast to prevent an empirical evaluation of mass transfer limitation (Joss et al., 2004; Plósz et al., 2010; Barret et al., 2011), molecular diffusion may have a major role in determining partitioning kinetics in biofilms. Biofilm characteristics such as biomass density and porosity have been found to influence intra-biofilm diffusion of a number of organic and inorganic chemical compounds. This effect has been described by introducing a coefficient f , defined as the ratio of effective diffusivity in biofilms and in free aqueous media, thus defining diffusivity reduction in biofilms (Fan et al., 1990; Guimerà et al., 2016; Horn and Morgenroth, 2006; Trapp and Matthies, 1998; Zhang and Bishop, 1994a). While f was determined for a number of organic and inorganic chemical compounds, no conclusive evidence currently exists for organic micropollutant diffusion in biofilms, which was therefore investigated in this study.

In our previous work (Torresi et al., 2016), we investigated the biological transformation of pharmaceuticals in nitrifying moving bed biofilm reactors (MBBRs) using novel MBBR carriers (AnoxKaldnes Z-carriers), allowing the control of the biofilm thickness.

In this study, the main objective set was to assess how the diffusion and partitioning of 23 selected pharmaceuticals vary at different biofilm thicknesses (50, 200 and 500 μm) and to quantify corresponding single point K_d values at environmentally relevant concentration levels. By developing and calibrating a model that describes diffusive transport and partitioning in biofilms, we aimed at elucidating the influence of biofilm thickness on (i) the molecular diffusion of micropollutants within biofilm matrix, described by the dimensionless effective diffusivity coefficient f ; (ii) the extent of partitioning, described by coefficient K_d . Additionally, using experimental and modelling results, the influence of biofilm characteristics (porosity, density) and molecular properties (e.g., hydrophobicity, ionization) on the mass

99 transfer limitation and sorption of micropollutants in biofilms were assessed.

2. Model development

2.1 Conceptual approach for diffusion and sorption in biofilms and model implementation

Considering molecular diffusion of dissolved micropollutants from the bulk aqueous phase into biofilms as the dominant mechanism (Zhang and Bishop, 1994a), the partitioning of organic micropollutants consists of three consecutive steps (Joss et al., 2004): (1) diffusion of dissolved micropollutant from bulk aqueous phase, through a boundary layer, into the biofilm matrix; (2) diffusion of dissolved micropollutant through the biofilm matrix via its pores; (3) sorption to the solid phase of the biofilm matrix (Fig. 1a). The diffusivity of organic chemicals in a free aqueous medium ($D_{w,i}$, $\text{m}^2 \text{d}^{-1}$) can be predicted from properties of the chemical (e.g., molar volume) and of the medium. In this study, $D_{w,i}$ values for each chemical were calculated according to Hayduk and Laudie (1974), although alternative approaches were also tested (Table S1 in Supplementary Information).

Transport from the bulk liquid to the biofilm is controlled by the diffusion through a boundary layer, for which the diffusivity was assumed equal to $D_{w,i}$ (**Assumption I**, Fig 1b). The thickness of the boundary layer, L_L (μm), was assumed to be equal to 10 μm for all the Z-carriers (Brockmann et al., 2008, see section 1 in SI). In biofilms, molecular diffusivity is reduced compared to free aqueous media (Wanner and Reichert, 1996). This has been attributed to the “tortuosity” of the transport path in biofilms, i.e. the increased (non-linear) path length needed for diffusive transport as compared to free aqueous media (Zhang and Bishop, 1994b). Molecular diffusivity reduction is described by the dimensionless coefficient f , resulting in Eq. 1:

$$D_{bf,i} = f \cdot D_{w,i} \quad (\text{Eq. 1})$$

where $D_{bf,i}$ ($\text{m}^2 \text{d}^{-1}$) is the effective diffusivity of micropollutants within biofilms and f (-) is always lower than 1. While f values of 0.5–0.8 have been assigned for micropollutant diffusion (Ort and Gujer, 2008; Vasiliadou et al., 2014), this parameter is likely to vary significantly depending on the biofilm structure and properties (biofilm thickness, density, porosity and tortuosity).

It has previously been shown that biofilm porosity and density can vary over the biofilm depth (Zhang and Bishop, 1994a). In the model, we assume the biofilm as a homogenous porous medium (**Assumption II**), although we accept that biofilms with different depth can have different average porosities and densities. As a consequence, only one f value was used to describe diffusion reduction into a biofilm with a certain thickness.

Sorption/desorption kinetics were described using first-order rate equations (see matrix in Fig. 1c). Sorption was considered as an equilibrium process (**Assumption III**), by attributing an arbitrarily high value to the desorption rate k_{des} , thereby making diffusion from the bulk aqueous phase and within the biofilm the rate-limiting steps for solid-liquid partitioning. At micropollutant concentration levels targeted in this study (ng L^{-1} to $\mu\text{g L}^{-1}$), sorption can be considered linear and better described by the distribution coefficient K_d (**Assumption IV**).

Based on the presented conceptual approach, a diffusion-sorption model was implemented as one-dimensional biofilm model in Aquasim 2.1 (Reichert, 1994). Design and measured biofilm properties (biofilm thickness, surface area, biomass density, porosity) were used as input to the model (see Table 1). Each biofilm was spatially discretized in 20 completely mixed layers. This allowed solving the generic mass balance equation for dissolved micropollutant concentration C_L (ng L^{-1}) in biofilm (Eq. 2):

$$\frac{\partial C_L}{\partial t} = D_{bf,i} \frac{\partial^2 C_L}{\partial z^2} - k_{des} K_d C_L X + k_{des} C_S \quad (\text{Eq. 2})$$

(where X is the biomass concentration in biofilm, g L^{-1} ; C_S is the sorbed micropollutant concentration, ng L^{-1} ; C_L varies with time t and depth z) as a set of ordinary differential equations by using the method of lines (Wanner and Reichert, 1996). According to the diffusion-sorption model, micropollutants undergo equilibrium microscale partitioning as they diffuse through biofilm, in analogy to the approach proposed by Wu and Gschwend (1986). Further details on the conceptual biofilm model, on microscopic mass balances and on the initial conditions are given in the Supplementary Information (section S1 and Figure S1).

< Figure 1 >

2.2 Calculation of sorption coefficients

At equilibrium, the micropollutant concentration sorbed onto biomass ($C_{S,eq}$, $\mu\text{g L}^{-1}$) is proportional to the dissolved concentration ($C_{L,eq}$, $\mu\text{g L}^{-1}$), and their ratio, normalized by the concentration of solids ($X_{biomass}$, g L^{-1}), is used to calculate the sorption coefficient $K_{d,eq}$ (L g^{-1}). With negligible transformation, it is commonly assumed (e.g., in activated sludge) that the sorbed concentration is equivalent to the decrease in dissolved concentration ($C_{L,0} - C_{L,eq}$) between the beginning and the end of batch sorption experiments.

When considering biofilm systems, transport in biofilm pores, along with sorption, can also determine a decrease of micropollutant concentrations in the bulk phase. Hence, the coefficient $K_{d,eq}$ (L g^{-1}) was defined to describe sorption in Z-carrier biofilms based on mass balance considerations (Eq. 3):

$$K_{d,eq} = \frac{\left[\frac{C_{L,0} V_{bulk}}{V_{bulk} + V_{bf,wet}} - \frac{C_{L,eq} (V_{bulk} + V_{PW})}{V_{bulk} + V_{bf,wet}} \right]}{C_{L,eq} X_{biomass}} \quad (\text{Eq. 3})$$

where V_{bulk} (L) denotes the volume of the bulk liquid, $V_{bf,wet}$ (L) the volume of wet biofilm (equal to the total surface area of Z-carriers times the defined biofilm thickness) and V_{PW} (L)

the volume of the pore water in the biofilm matrix, not accounting for cellular water content (see 3.4). The procedure used to derive Eq. 3 is presented in detail in the Supplementary Information (section S3).

The ‘asymptotic’ concentration $C_{L,eq}$, defining true sorption equilibrium, was estimated by fitting measured concentration profiles in batch sorption experiments with a first-order decay equation (Eq. 4)

$$C_L(t) = (C_{L,0} - C_{L,eq})e^{-kt} + C_{L,eq} \quad (\text{Eq. 4})$$

In activated sludge, it has been widely accepted that sorption equilibrium can be reached within 0.5–1 h (Ternes et al., 2004; Andersen et al., 2005; Yi and Harper, 2007, Hörsing et al., 2011). To verify whether sorption equilibrium was achieved relatively fast (i.e., within the 4-hour duration of sorption experiments) also in Z-carrier biofilms, the sorption coefficient $K_{d,4h}$ ($L\ g^{-1}$) was calculated (Eq. 5):

$$K_{d,4h} = \frac{\left[\frac{C_{L,0}V_{bulk}}{V_{bulk} + V_{bf,wet}} - \frac{C_{L,4h}(V_{bulk} + V_{PW})}{V_{bulk} + V_{bf,wet}} \right]}{C_{L,4h}X_{biomass}} \quad (\text{Eq. 5})$$

where $C_{L,4h}$ is the measured dissolved concentration in bulk aqueous phase at $t=4$ h (the last measurement in sorption experiments), replacing $C_{L,eq}$ in Eq. 3. Specifically, the 4-hour equilibrium assumption was verified by comparing $K_{d,4h}$ and $K_{d,eq}$ and assessing the relative deviation between the two coefficients.

As mentioned above, the decrease of bulk micropollutant concentration during sorption experiments with biofilms results from transport in biofilm pores (besides sorption in biofilms). To verify the impact of neglecting mass transfer to biofilm pores on sorption coefficient determination, the sorption coefficient $K_{d,susp}$ was calculated (Eq. 6):

$$K_{d,susp} = \frac{C_{L,0} - C_{L,eq}}{C_{L,eq}X_{biomass}} \quad (\text{Eq. 6})$$

where $C_{L,eq}$ was calculated using Eq. 4. Notably, Eq. 6 is commonly used to describe sorption onto suspended activated sludge, where the effect of porosity is neglected. The comparison between $K_{d,eq}$ and $K_{d,susp}$ (together with relative deviation the two coefficients) was used to quantify the contribution of transport to biofilm pores, hence the impact of porosity, on the estimated sorption coefficient.

2.3 Parameter estimation approach

The assessment of diffusion and sorption of micropollutants in biofilms consisted of two main consecutive steps performed for each micropollutant and at different biofilm thicknesses: (i) calculation of the coefficient $K_{d,eq}$ (section 2.2); (ii) calibration of the diffusion-sorption model (section 2.1) against experimental data and estimation of the coefficient f , which was the only parameters fitted in the model. Estimation of f was performed using the secant model calibration algorithm embedded in Aquasim 2.1.

3. Materials and methods

3.1. System description and operation

Nitrifying MBBRs used in this study have been described elsewhere (Torresi et al., 2016). Briefly, two laboratory-scale nitrifying MBBRs were operated in parallel under continuous-flow conditions for approximately 300 days. Z-carriers (AnoxKaldnes AB, Lund, Sweden) were used to obtain biofilm of different thicknesses. Z-carriers have a saddle shaped grid covered surface allowing for biofilm growth only up to the height of the grid wall (Torresi et al., 2016). Three different Z-carriers (named Z50, Z200, and Z500) were used in this study, with the numbers indicating the grid wall height in μm (hence the maximum controlled biofilm thickness). Biofilms were enriched by feeding the MBBRs with effluent wastewater from a local municipal treatment plant (Källby, Lund, Sweden), spiked with ammonium (50 mg L^{-1} of $\text{NH}_4\text{-N}$ as NH_4Cl) and phosphate (0.5 mg L^{-1} of $\text{PO}_4\text{-P}$ as KH_2PO_4). The MBBRs were operated under similar conditions, i.e. hydraulic residence time of 2 h, dissolved oxygen concentration of $4.5 \pm 0.5 \text{ mg L}^{-1}$, pH of 7.5 ± 0.5 and temperature of 20°C (achieved using a thermostat).

3.2. Sorption batch experiments

Sorption batch experiments were performed after reaching stable nitrogen removal (Torresi et al., 2016), roughly, around day 300. Prior to batch experiments, the two MBBRs were disconnected and three types of Z-carriers (Z50, Z200, Z500) were manually separated. Subsequently, Z-carriers were left overnight at 4°C in a beaker with tap water to allow for desorption of micropollutants possibly sorbed during continuous-flow operation.

Sorption batch experiments were carried out in three 200-mL glass beakers using filtered ($0.2 \mu\text{m}$ Munktell MG/A glass fiber filters) effluent wastewater from Källby treatment plant. Ammonium and nitrate in the feed were at concentration of $<0.5 \text{ mgN L}^{-1}$ and 6 mgN L^{-1} ,

respectively, while organic carbon concentration was lower than 35 mgCOD L^{-1} , mostly in inert form.

The biomass concentration in the three glass beakers was adjusted to 0.8 g L^{-1} based on attached biomass concentration measurements for the different carriers and adjusting the number of carriers accordingly (56 carriers for Z50, 32 for Z200 and 16 for Z500), resulting in a total biofilm surface area of 0.06, 0.04, 0.02 m^2 for the batch containing Z50, Z200 and Z500 carriers, respectively. Other abiotic removal processes, such as volatilization, sorption of micropollutants on plastic carriers and glass wall, had been previously assessed and found negligible in MBBRs (Torresi et al., 2016).

Twenty-three micropollutants were spiked in all the beakers with an initial concentration of $1 \mu\text{g L}^{-1}$ except for X-ray contrast media ($15 \mu\text{g L}^{-1}$), as they are usually found at higher concentrations in effluent wastewater (Margot et al., 2015). A stock solution, containing micropollutants dissolved in methanol (40 mg L^{-1}), was first spiked into empty glass beakers and the methanol was allowed to evaporate in the fumehood for approximately 1 hour. Subsequently, the solution was resuspended in filtered effluent for approximately 30 min to dissolve the spiked micropollutants. Biomass inactivation was achieved by: (i) addition of allylthiourea (ATU, 10 mg L^{-1} , Tran et al., 2009; Khunjar and Love, 2011) and nitrogen sparging (Hamon et al., 2014) to inhibit nitrifying bacteria; and (ii) addition of sodium azide (0.5 g L^{-1} ; Rattier et al., 2014) to inhibit the activity of heterotrophic bacteria.

The experiment duration was set to 4 hours. Homogenous aqueous samples were collected at regular intervals from the bulk phase in each beaker at 0, 5, 10, 30, 90 and 240 min. The batch experiments were performed at ambient temperature and initial pH was measured to be 7.5 ± 0.5 . Since only one spiking concentration was tested, results from sorption experiments were used to determine single point K_d values.

3.3. Chemicals

Twenty-three environmentally relevant micropollutants were selected for this study. The targeted pharmaceuticals were grouped in six categories according to their use: (i) four beta-blocker pharmaceuticals (atenolol, metoprolol, propranolol and sotalol); (ii) five X-ray contrast media (diatrizoic acid, iohexol, iopamidol, iopromide, iomeprol); (iii) three sulfonamide antibiotics (sulfadiazine, sulfamethizole and sulfamethoxazole), one metabolite (acetyl-sulfadiazine) and one combination product (trimethoprim); (iv) three non-steroidal anti-inflammatory pharmaceuticals (phenazone, diclofenac, ibuprofen); (v) three psycho-active drugs (carbamazepine, venlafaxine and citalopram); (vi) three macrolide antibiotics (erythromycin, clarithromycin and roxithromycin). Further information regarding chemical structure and properties, CAS numbers and chemical suppliers can be found in Table S2–S3 and in Escolà Casas et al. (2015).

3.4. Analytical methods

Samples for micropollutant analysis were collected (4 mL) and analysed via direct injection using internal standards (injected volume of 100 µL). Details regarding sample preparation, internal standards, HPLC and mass spectrometry conditions, limits of detection and quantification are shown in Escolà Casas et al. (2015). Biomass concentration on Z-carriers was measured in two ways: (i) as attached biomass concentration (expressed as total attached solids, TAS), calculated from the difference in weight of three dried carriers (105°C for > 24 h) before and after biofilm removal (using 2M H₂SO₄ with subsequent brushing) (see also Escolà Casas et al., 2015; Falås et al., 2013; Torresi et al., 2016); and (ii) by scraping and suspending the biofilm in tap water and measuring total suspended solids (TSS) and volatile suspended

solids (VSS) according to APHA standard methods (Clesceri, 1989). Biofilm properties such as biofilm dry density ρ_d (g cm^{-3}), biomass density in wet biofilm ρ (kg m^{-3}) and porosity ε (%) were calculated according to Tchobanoglous et al. (2003) and Hu et al. (2013) using measured biofilm properties (e.g., solids content), as detailed in the Supplementary Information (section S2). Porosity is defined as the fraction of the biofilm volume occupied only by water outside the cells and not inside the cells (Hu et al., 2013). Furthermore, ρ_d denotes the dry mass of biofilm per volume of dry biofilm (i.e., defines a *true* density) while ρ denotes the dry mass of biomass per volume of wet biofilm (i.e., defines a concentration of biomass within the biofilm). Further discussion on the calculation methodology used and on the biofilm properties can be found in section S2.

3.5. Statistical analysis and influence of chemical properties

Pearson's and Spearman's correlations between $K_{d,eq}$ and chemical properties (expressed in logarithmic base) were assessed at different biofilm thicknesses. A significance level of 0.05 was used for all statistical tests in this study. The investigated physico-chemical properties include: the molecular volume MV ($\text{cm}^3 \text{mol}^{-1}$); the dissociation constant(s) pK_a ; the number of rotatable bonds (nRB); the van der Waals area ($vdWA$, $\text{m}^2 \text{kmol}^{-1}$) (Sathyamoorthy and Ramsburg, 2013); McGowan's approximation of the molecular volume (V_X , $\text{cm}^3 \text{mol}^{-1}$) (Droge and Goss, 2013a); and the topological polar surface area ($TPSA$, \AA^2) (Ertl et al., 2000). Chemical properties and $K_{d,eq}$ were log transformed (Vasudevan et al., 2009) with exception of nRB (Sathyamoorthy and Ramsburg, 2013) and molecular size descriptors MV and V_X . Chemical properties for each compound were retrieved using ACD/Labs predictions and the database Mol-inisticts (for $\log vdWA$) or calculated based on previously defined equations (for V_X : Abraham and McGowan, 1987; Droge and Goss, 2013a). Pearson's and Spearman's

300 correlations and their significance were assessed using GraphPad Prism 5.0. Furthermore,
301 possible correlations between f and the abovementioned properties were also investigated.
302 Significant differences between estimated f values for each chemical at different biofilm thickness
303 were determined by examining the overlap between standard deviations of the estimate (Cumming
304 et al., 2007)

4. Results and discussion

4.1. Biofilm properties

Measured and calculated values for a number of biofilm properties are reported in Table 1. Dry biofilm mass per surface area of carrier (gTAS m^{-2} , Table 1) increased with biofilm thickness, being approximately four times higher in Z500 compared to Z50. Biofilm thickness in Z-carriers was recently measured using optical coherence tomography (OCT), revealing good agreement between measured and nominal thickness based on carrier design (Piculell et al., 2016). Conversely, biofilm density in wet biofilm ρ (section 1 in SI) in Z50 was up to 3-fold higher as compared to Z200 and Z500. This suggests a change in biofilm porosity as a function of biofilm thickness. Biofilm porosity ε (Eq. S12), ranged from 75% (Z50) to 93% (Z500) (Table 1). An approximate porosity of 80% is commonly assumed in one-dimensional biofilm models (Wanner and Reichert, 1996; Brockmann et al., 2008) and similar values have been previously determined using modelling approximations (Zacarias et al., 2005; Zhang and Bishop, 1994b). The observed increasing ε with biofilm thickness is in agreement with previous findings for Z-carrier biofilms (Piculell et al., 2016), although lower porosities (approximately of 10 and 30% for Z50 and Z400) were estimated using OCT. Values of biofilm dry density ρ_d (Table 1) for the three biofilms were comparable to that shown in literature (Hu et al., 2013), indicating a higher content of fixed solids in Z500.

< Table 1 >

4.2. Sorption coefficients in biofilms

Sorption was considered significant when a relative concentration drop $(C_{L,0} - C_{L,4h})/C_{L,0}$ higher than 10% was observed (Hörsing et al., 2011), thus accounting for analytical

uncertainty. Profiles of aqueous concentration of the sorptive micropollutants measured during batch experiments are shown in Fig. 3 (duplicate measurement) and in Fig. S2.

Out of the 23 targeted compounds, sorption was significant only for eight micropollutants, namely atenolol, metoprolol, propranolol, citalopram, venlafaxine, erythromycin, clarithromycin and roxithromycin. The presence of chemicals not exhibiting sorption (e.g., diclofenac and the targeted sulfonamides) suggests that biomass was successfully inhibited during batch experiments, as most targeted compounds were significantly biodegraded in the same MBBRs without biomass inhibition (Torresi et al. 2016). Interestingly, micropollutants that were positively charged (>90% cationic fraction) at the experimental pH of 7.5 presented significant sorption, with exception of sotalol and trimethoprim. Higher sorption potential of positively charged compounds compared to negatively charged or neutral compounds was previously observed for activated sludge biosolids (Stevens-Garmon et al., 2011; Polesel et al., 2015) and soil (Franco and Trapp, 2008).

4.2.1. Sorption coefficients $K_{d,eq}$ and comparison with activated sludge

Sorption coefficients $K_{d,eq}$ in Z50, Z200 and Z500 biofilms were calculated for the above listed cationic micropollutants (Table 2). $K_{d,eq}$ values were compared with previously found sorption coefficients in activated sludge, for which the large majority of micropollutant sorption data are available.

< Table 2 >

Values of $K_{d,eq}$ for atenolol at all the three biofilm thickness were up to 2-fold higher than literature values for activated sludge (Radjenović et al., 2009; Stevens-Garmon et al., 2011), while values in Z50 and Z200 were comparable with findings for secondary sludge (Hörsing et al., 2011). As atenolol presents similar molecular properties to other beta-blockers (e.g.,

352 molecular weight, pK_a), the reasons behind this high sorption potential are unclear. As to
 353 metoprolol, $K_{d,eq}$ values in Z50, Z200 and Z500 were comparable to previously measured
 354 coefficients in activated sludge biomass (Maurer et al., 2007; Sathyamoorthy et al., 2013).
 355 Similarly to studies on sludge, propranolol exhibited the highest sorption potential of all
 356 selected beta-blockers (Maurer et al., 2007; Radjenović et al., 2009). Notably, a fourth targeted
 357 beta blocker sotalol did not show any significant sorption, in agreement with previous findings
 358 in activated sludge (Maurer et al., 2007; Sathyamoorthy et al., 2013).
 359 Values of $K_{d,eq}$ for Z50 and Z200 were comparable with previous studies on conventional
 360 activated sludge and membrane bioreactor (MBR) sludge for clarithromycin (Abegglen et al.,
 361 2009; Göbel et al., 2005), erythromycin (Radjenović et al., 2009; Xue et al., 2010) and
 362 roxithromycin (Abegglen et al., 2009; Hörsing et al., 2011). On the contrary, $K_{d,eq}$ for Z500
 363 differed by one order of magnitude from previously reported values. Nevertheless, 50–80% of
 364 dissolved clarithromycin and roxithromycin sorbed on MBR sludge (Abegglen et al., 2009),
 365 similarly to clarithromycin and erythromycin in this study (~80%). Furthermore, highly
 366 variable macrolide sorption was shown in soil and onto humic acids (Sibley and Pedersen,
 367 2008; Uhrich et al., 2014), with estimated $K_{d,eq}$ values also higher than 8 L g⁻¹ or 20 L g⁻¹,
 368 respectively. Macrolides exhibited the highest $K_{d,eq}$ of all sorptive compounds in Z500 but not
 369 at lower biofilm thickness (Table 2). This might be related to the low porosity of the biofilms
 370 Z50 and Z200. According to Lipinski's rule of five (Lipinski et al., 1997), macrolides are
 371 expected to poorly permeate across cell membranes and thus to move only in the intracellular
 372 space (depending on the porosity) due to their high molecular weight (>500 g mol⁻¹).
 373 Furthermore, macrolides are mainly excreted in feces (Göbel et al., 2005) and due to
 374 protonation of the tertiary amino group, strong ionic interaction of macrolides with the
 375 negatively charged surface of the biomass could be expected.

Few studies investigated the sorption of the antidepressant venlafaxine and the antiepileptic citalopram. While sorption coefficients for Z50 and Z200 for both compounds are in agreement with existing literature on activated sludge (Hörsing et al., 2011), higher values were found in Z500 for citalopram.

In general, sorption coefficients of all the compounds at the three biofilm thicknesses were comparable or higher than values observed with activated sludge biomass. Studies comparing sorption onto MBR sludge and conventional activated sludge biomass (Joss et al., 2006; Abegglen et al., 2009; Reif et al., 2011; Yi and Harper, 2007) revealed a sorption enhancement in the former case. Increased sorption was associated to the smaller size of MBR sludge flocs (assumed to be around 80–300 μm in diameter), thus resulting in higher accessible surface area (Tchobanoglous et al., 2003). In analogy with MBR sludge, it can be postulated that the high accessible surface area in Z-carrier biofilms (related to the biofilm porous structure) may explain the increased sorption capacity of most of the compounds compared to conventional activated sludge biomass.

4.2.2. Comparison between $K_{d,eq}$ and $K_{d,4h}$

Sorption coefficients $K_{d,eq}$ were compared with $K_{d,4h}$ values for each chemical and relative deviations Δ (%) between these two coefficients were calculated at different biofilm thicknesses (Table 2) to verify the equilibrium assumption within the experiment duration (4 hours). For most compounds, relative deviations for Z50 and Z200 were on average around 10%, with the exception of atenolol (>50%). Conversely, Δ values in Z500 were for five compounds higher than 30% (up to 80% for atenolol).

Overall, while the assumption of equilibrium reached within 4 h seems justified for Z50 and Z200, diffusive mass transfer can significantly influence observations at higher biofilm

thickness. Atenolol was the main exception, for which the 4-h equilibrium assumption seems not valid at any biofilm thickness. On the contrary, propranolol appeared to reach partitioning equilibrium within 4-h in Z50, Z200 and Z500, and similar considerations could be made for citalopram and venlafaxine. Therefore, to reduce uncertainties in sorption experiments, parameter estimation can benefit from calculating the asymptotic aqueous concentration value using e.g., simplified first-order decay equations (Eq. 4).

4.2.3. Comparison between $K_{d,eq}$ and $K_{d,susp}$ and trends with biofilm thickness

To assess the impact of biofilm porosity and mass transfer in pores on sorption coefficient estimation, the sorption coefficients $K_{d,eq}$ and $K_{d,susp}$ were compared (Table S4). In Fig. 2, this comparison is presented for two key chemicals (a: metoprolol, b: roxithromycin). For all micropollutants, neglecting the transport from bulk aqueous phase to biofilm pores resulted in an overestimation of sorption coefficients ($K_{d,susp}$ always greater than $K_{d,eq}$). The relative deviation between $K_{d,susp}$ and $K_{d,eq}$ was on average $\leq 10\%$ for most compounds and 30% for less sorptive compounds (metoprolol and venlafaxine).

We further observed that both $K_{d,eq}$ and $K_{d,susp}$ generally increased with increasing biofilm thickness (Fig. 2). Specifically, $K_{d,eq}$ values in Z500 were from 4-fold (most of the compounds) up to 30-fold higher (macrolides antibiotics) than in Z50 (Table 2). It should be highlighted that batch experiments were carried out at the same biomass concentration in the reactors (0.8 g L⁻¹). Consequently, the observed $K_{d,eq}$ increase with biofilm thickness likely derives from differences in biofilm composition and/or in its physical properties. Two possible explanations of this observation were proposed:

(i) Biomass composition, such as the relative fraction of autotrophic and heterotrophic bacteria and/or the content of extracellular polymeric substances (EPS), can influence sorption

properties. EPS protein content was previously positively correlated with K_d for aromatic chemicals in untreated and treated sewage sludge and colloids (Barret et al., 2010) and for the estrogen EE2 and trimethoprim in nitrifying and heterotrophic biomass (Khunjar and Love, 2011). Bassin et al., (2012) further observed higher concentration of proteins and polysaccharides (that mainly compose EPS) in heterotrophic MBBRs than in nitrifying MBBRs. Higher fractions of heterotrophic bacteria (determined using quantitative PCR of 16S rRNA) were measured in Z200 and Z500 compared to Z50 (Torresi et al., 2016), possibly justifying the increased sorption capacity in thicker biofilms (Z200, Z500). Further investigation on the EPS content in the different biofilms is thus required to support this hypothesis, given the key role of EPS in the sorption of neutral and ionizable organic chemicals (Späth et al., 1998; Barret et al., 2010; Khunjar and Love, 2011).

(ii) Porosity can influence the available surface area inside the biofilm. Sorption has been previously positively impacted by reduced particle size, i.e., greater surface area, in suspended biomass (Khunjar and Love, 2011) and biomass floc suspension derived from MBRs (Yi and Harper, 2007). Thicker biofilms, having lower biomass density and substantially higher porosity than thin biofilms, could accordingly provide for higher available surface (and thus more accessible sites) for solid-liquid partitioning.

Finally, $K_{d,eq}$ values were normalized to the highest value of $K_{d,eq}$ (i.e., for Z500, $K_{d,eqZ500}$). The obtained profiles followed two distinct trends as a function of biofilm thickness (Fig. 2c–d): (i) beta-blockers and venlafaxine, exhibiting a logarithmic-like increase between Z50 and Z500; and (ii) macrolides and citalopram, presenting significantly higher values for Z500, thus an exponential-like increase of $K_{d,eq}$ with thickness. The question arises as to the influence of the specific chemical properties of micropollutants on partitioning in biofilms, which was further assessed using correlation analysis (see 4.5.2).

< Figure 2 >

4.3. Modelling diffusion and sorption in biofilm

Based on the considerations above, calculated $K_{d,eq}$ were used to calibrate the diffusion-sorption model against experimental data for the estimation of the dimensionless effective diffusivity coefficient f (the only parameter estimated with the model). Simulated aqueous concentrations (continuous lines, Fig. 3) predicted reasonably well the measured concentrations in bulk liquid (circles, Fig. 3) for most of the targeted compounds (i.e., for propranolol, clarithromycin, erythromycin, roxithromycin, citalopram, venlafaxine $R^2 > 0.9$; Table S5). For atenolol, measured concentrations were less well predicted for Z50 and Z500 (R^2 equal to 0.8).

The simulated micropollutant concentrations in the bulk liquid and in the biofilm pores liquid (dashed lines, Fig. 3) should converge when partitioning equilibrium is reached. This equilibrium condition was satisfied for most compounds in Z50 and Z200 within 4 h experimental time, with an average 10% relative deviation between simulated concentrations in bulk and in biofilm pores. For the thickest biofilm (Z500), however, model predictions for most of targeted chemicals suggested that equilibrium was not reached within 4 h (60% average discrepancy with the last measurement). It is likely that, due to the greater thickness, increased time to diffuse in deeper biofilm and thus to achieve sorption equilibrium is required in Z500. The exception was propranolol, for which equilibrium seemed to be reached in all the three biofilms, thus supporting results (relative deviation between $K_{d,4h}$ and $K_{d,eq}$) presented in Table 2. For macrolide antibiotics, this discrepancy was significant and simulation results suggested a time for partitioning equilibrium of approximately 10 days—in good agreement with equilibrium times (days, months and years) in other environmental matrices (Delle Site,

2001). Furthermore, the large molecular volume and weight of macrolides (2- to 3-fold higher than the other targeted compounds, Table S2), as well as their high sorption potential in Z500, suggest slower diffusive transport inside the biofilm, as previously observed for hydrophobic organic molecules in sediments and soil (Wu and Gschwend, 1986).

There is a large variability concerning the time to reach partitioning equilibrium for organic chemicals in biofilms (Alvarino et al., 2015; Headley et al., 1998; Shi et al., 2011; Wicke et al., 2008; Writer et al., 2011), with values ranging from, e.g., 4 to 80 h for biofilm of 0.1 mm thickness (Wicke et al., 2008). In conclusion, our observations conflict with the widely held assumption of significantly shorter period of time (i.e. minutes to 1–2 hours) necessary to reach equilibrium in activated sludge (e.g., Hörsing et al., 2011; Pomiès et al., 2013). This may be explained by differences in pore-scale (hydro)dynamic conditions in MBBRs and activated sludge reactors, resulting in more pronounced mass transfer limitation in MBBRs.

< Figure 3 >

4.4. Influence of biofilm and chemical properties on diffusion (f) and partitioning ($K_{d,eq}$)

4.4.1. Estimation of f and proposed empirical correlation

Values of the dimensionless effective diffusivity coefficient f estimated for the three biofilm thicknesses and the eight sorptive compounds are reported in Fig. 4. For most of the compounds, with the exception of roxithromycin, f decreased with biofilm density and thus increased with biofilm thickness and porosity (with f in Z500 significantly higher than in Z200 and Z50 for all the compounds, and f in Z200 significantly higher than Z50 for six compounds). In thinner biofilms ($\leq 50 \mu\text{m}$), the transport of micropollutants could thus be limited by the high biomass density and the reduced porosity. A number of regressions to estimate f of solutes in biofilms as a function of biofilm density or porosity have been

previously developed (Fan et al., 1990; Guimerà et al., 2016; Horn and Morgenroth, 2006; Zhang and Bishop, 1994a), suggesting a negative correlation between f and density. Selected regression profiles (i.e., Guimerà et al., 2016; Horn and Morgenroth, 2006; Zhang and Bishop, 1994a; see Table S6) are reported in Fig. S3 for comparison with our f estimations. In particular, Guimerà et al. (2016) observed strong mass transfer limitation ($f < 0.1$) for oxygen in biofilm with density greater than 50 gVSS L⁻¹, in close agreement with findings (specifically for Z50) presented in this study.

< Figure 4 >

In general, estimated f were lower than values calculated from proposed regressions (Guimerà et al., 2016; Horn and Morgenroth, 2006; Zhang and Bishop, 1994a) (Fig. S3). While these regressions were identified for solutes with lower molecular weight (< 100 g mol⁻¹) and high solubility (e.g., O₂, sodium chloride, sodium nitrate), lower values of f (~ 0.2) were reported for most organic solutes with larger molecular weight (e.g., sugars and fatty acids; Stewart, 2003, 1998).

Given the possible influence of chemical properties on micropollutant diffusivity, we evaluated the relationship between f and several physico-chemical descriptors (section 3.5). No specific correlation was observed between f and molecular volume and other descriptors (Fig. S4). We observed a positive correlation only between f and $\log K_{OW}$ of the targeted compounds (Fig. S5), while negative dependence was reported in literature for organic compounds (Headley et al., 1998; Wicke et al., 2007; Wu and Gschwend, 1986). Notably, in this study the correlation was found for less hydrophobic ($0.1 < \log K_{OW} < 3.7$) and positively charged compounds (differently from previous studies), for which electrostatic interactions may also have influenced transport and partitioning. Thus, an empirical correlation between f , biofilm density ρ (as function of biofilm thickness) and $\log K_{OW}$ is proposed (Eq. 7):

$$f = \frac{1}{488 \cdot e^{-0.0072L_F}} \ln \left(\frac{-127 - \log K_{OW,max}}{\log K_{ow} - \log K_{OW,max}} \right) \quad (\text{Eq. 7})$$

where L_F is the biofilm thickness (μm) and $\log K_{OW,max}$ is the asymptotic $\log K_{OW}$ approximating the highest value for the compounds selected. Profiles of f deriving from Eq. 7 were then depicted in Fig. 5, along with the estimated f values for the three biofilm thickness (symbols, see also Fig. 4). Further details on the formulation of Eq. 7 are given in the SI (section S4). We note that the size of the available data set may not be sufficiently large to validate the correlation, and additional experimental evidence (higher biofilm thickness, wider range of $\log K_{OW}$) may be required for further confirmation.

< Figure 5 >

4.4.2. Predictors of micropollutant $K_{d,eq}$ in biofilms

Correlation analyses were performed between $K_{d,eq}$ and a number of physico-chemical micropollutant descriptors.

First, the octanol-water partitioning coefficient of the neutral species ($\log K_{OW}$) and the species-dependent octanol-water distribution coefficient ($\log D$) were assessed, exhibiting insignificant correlation with $K_{d,eq}$ ($-0.27 < \text{Pearson's } r < 0.15$ for the three biofilms). This finding confirms the limited reliability of $\log K_{OW}$ and $\log D$ as sorption predictors for organic cations, as previously shown in soil (Tolls, 2001; Franco and Trapp, 2008; Droge and Gross, 2013a).

Following this preliminary assessment, correlations with physico-chemical descriptors for ionizable compounds (i.e., $\log pK_a$, nrB , MV , $\log TPSA$, $\log vdwA$, V_X) were investigated (Fig. 6 and S6). Correlations for biofilm Z50 was performed only considering six compounds ($K_{d,eq} = 0$ for venlafaxine and roxithromycin).

No significant correlations were found with the stereochemistry parameter nRB and $\log pK_a$ (Fig.S6). While previous studies positively correlated the sorption of cationic compounds with pK_a ($r^2=0.5$) (Franco and Trapp, 2008), the narrow range of pK_a values covered in this study prevented us from concluding on the significance of this indicator.

Interestingly, our analysis revealed a significant positive correlation only for Z500 between $\log K_{d,eq}$ and $\log TPSA$, $\log vdWA$, McGowan's V_X (Fig. 6) and MV (Fig. S6a). The parameter $TPSA$ was previously identified as sorption predictor only for neutral and negatively charged compounds, although with a negative correlation (Sathyamoorthy and Ramsburg, 2013). $TPSA$ reflects the polarity of the organic chemical by accounting for the oxygen and nitrogen atoms as well as attached hydrogen atoms, and increased polar surface area has been associated to reduced absorption and cell permeability of pharmaceuticals in humans (Palm et al., 1997; Ertl et al., 2000). Hence, the significant correlation with $\log K_{d,eq}$ may suggest (at least for thicker biofilm) a positive influence of polarity on the retention of cations in biofilm, possibly resulting from the improved accessibility to deeper biofilm through transport in the intracellular space.

On the other hand, the positive correlation of $\log K_{d,eq}$ with $\log vdWA$, MV and V_X still suggests a contribution of hydrophobicity in sorption of positively charged compounds in Z500 biofilm. This finding is in line with previously established regressions for the prediction of distribution coefficients based on van der Waals volume (Kamlet et al., 1998) or V_X (Abraham, 1993; Abraham and Acree, 2010; Droge and Goss, 2013a,b,c) for neutral and ionized molecules. Notably, McGowan's volume positively correlates with van der Waals volume (Zhao et al., 2003), which is itself correlated to $vdWA$. Hence, both $vdWA$ and V_X provide an indication of the influence of the molecular size in the cavity formation mechanism, through which solute

molecules can distribute to an organic phase at the expenses of (i.e., by replacing) water molecules (Mackay and Vasudevan, 2012).

Considering the relevance of the correlation between $\log K_{d,eq}$ and V_X for Z500, an empirical regression model (Eq. 8) was tested based on the equation previously proposed by Droge and Goss (2013a,c) for sorption prediction of organic cations to soil organic matter:

$$\log K_{d,eq} = a \cdot V_X / 100 + b \cdot NA_i + c \quad (\text{Eq. 8})$$

where $K_{d,eq}$ is expressed in L kg^{-1} and NA_i indicates the number of hydrogen atoms bound to the charged nitrogen moiety. The coefficients a , b and c were estimated by fitting Eq. 8 to measured sorption coefficients. The comparison between predicted and measured $\log K_{d,eq}$ for Z500 is shown in Fig. 6d ($a=0.35$; $b=0.45$; $c=1.48$). The regression ($r^2 = 0.58$) could only partly describe sorption of cationic micropollutants in Z500 biofilms, yielding rather good $K_{d,eq}$ predictions (within factor 1.5 from measurements) for propranolol, clarithromycin, erythromycin and roxythromycin. Potential improvement of sorption predictions may be expected from the identification of correction factors for polar functional groups—an area beyond the scope of this study due to the limited number of substances.

Overall, results from this assessment confirm the challenges in the identification of unique and reliable sorption predictors for positively charged micropollutants in biofilm, as previously recognized for other matrices (Kah and Brown, 2007; Franco and Trapp, 2008; Franco et al., 2009; Sathyamoorthy and Ramsburg, 2013; Droge and Goss, 2013a,c; Bittermann et al., 2016). Nevertheless, it should be highlighted that in this study sorption was consistently observed only for positively charged compounds, indicating that electrostatic interaction with negatively charged biomass surfaces play a major role for sorption in biofilms.

< Figure 6 >

5. Conclusions

This study investigated the sorption and the diffusion of selected micropollutants in nitrifying MBBR biofilms (thickness=50, 200, 500 μm) by means of targeted experiments and process modelling, leading to the following conclusions:

- Sorption in biofilm occurred only for eight positively charged micropollutants (i.e., three macrolides, three beta-blockers and two psycho-active pharmaceuticals) out of 23 targeted substances. Electrostatic interaction with the negatively charged biomass surfaces appears to play a major role in the sorption to biofilms.
- Values of the partitioning coefficient $K_{d,eq}$ increased with increasing biofilm thickness for most of the sorbed compounds, being related to the increasing biofilm porosity and thus the higher surface area accessible for sorption. Sorption equilibria were reached within the duration of sorption experiments (4 h) for a number of compounds in 50 and 200 μm thick biofilms, but not in the thickest biofilm. Slower equilibrium in thick biofilms ($\geq 500 \mu\text{m}$) is likely determined by the longer time required to diffuse in deeper biofilm.
- Dimensionless effective diffusivity coefficients f for micropollutants (estimated for the first time in wastewater treatment biofilms) were negatively correlated with biofilm density, while showing an increase with increasing porosity. This indicates that diffusive transport may be strongly limited by the higher biomass density (and the lower porosity) of thinner biofilms.
- Significant positive correlations were observed between $\log K_{d,eq}$ and a limited number of chemical properties of micropollutants (topological polar surface area, van der Waals area and McGowan's volume) but not for all biofilm thicknesses, confirming the challenges in the prediction of sorption in biofilms and other matrices for positively

charged compounds.

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Table 1. Biofilm characteristics and input parameters used in the sorption and diffusion model in this study. The parameter ρ_d denotes the dry mass of biofilm per volume of dry biofilm (defining a true density), while ρ denotes the dry mass of (microbial) biomass per volume of wet biofilm (defining the concentration of biomass within the biofilm). TAS defines total attached solids.

Parameter	Z50	Z200	Z500	Reference
Dry biofilm mass per carrier (gTAS m ⁻²)	2.6 ± 0.2	4.0 ± 0.3	8.0 ± 0.6	Measured
Biofilm dry density ρ_d (g cm ⁻³)	1.05 ± 0.09	1.05 ± 0.07	1.17 ± 0.05	Calculated
Biomass density in wet biofilm ρ (kg m ⁻³)	51.9 ± 2.6	20.0 ± 1.3	16.0 ± 0.8	Calculated
Porosity ε (%)	75 ± 4	91 ± 6	93 ± 7	Calculated
Biomass concentration in batch reactor (gTAS L ⁻¹)	0.80 ± 0.07	0.78 ± 0.06	0.78 ± 0.03	Measured

Table 2. Sorption coefficients calculated using the asymptotic equilibrium concentration ($K_{d,eq}$, L g⁻¹; mean and standard deviation are given) and the last measured aqueous concentration (t=4 h) during batch experiments ($K_{d,4h}$, L g⁻¹) for eight of the 23 spiked chemical compounds. The parameter Δ (%) defines the relative deviation between the two K_d values, providing also an indication of the deviation from partitioning equilibrium. Literature K_d values comprise measured partition coefficients in conventional activated sludge and membrane bioreactor (MBR) sludge.

	Z50			Z200			Z500			Literature K_d (L g ⁻¹)
	$K_{d,eq}$	$K_{d,4h}$	Δ	$K_{d,eq}$	$K_{d,4h}$	Δ	$K_{d,eq}$	$K_{d,4h}$	Δ	
	(L g ⁻¹)	(L g ⁻¹)	(%)	(L g ⁻¹)	(L g ⁻¹)	(%)	(L g ⁻¹)	(L g ⁻¹)	(%)	
Atenolol	1.12±2.21	0.26	77	1.12±0.34	0.68	38	4.84±0.73	0.95	80	(0.006) ¹ –1.9 ²
Metoprolol	0.08±0.01	0.08	3	0.19±0.06	0.16	15	0.28±0.02	0.15	44	<0.01 ³ –0.23 ⁴
Propranolol	0.50±0.04	0.54	-9	1.71±0.03	1.67	1	1.95±0.06	1.92	-1	0.2 ¹ –0.32 ³
Clarithromycin	0.42±0.11	0.34	20	0.41±0.02	0.39	4	11.19±0.20	5.63	48	0.26 ⁵ –1.2 ⁶
Erythromycin	0.33±0.07	0.34	-3	0.20±0.01	0.20	-4	11.28±2.10	6.13	43	0.31 ¹ –1.0 ⁷
Roxithromycin	0.00	0.00	/	0.86±0.13	1.05	-24	11.10±0.30	3.92	64	<0.1 ⁸ –0.5 ⁶
Citalopram	0.47±0.08	0.46	1	0.67±0.08	0.61	8	2.52±0.15	2.06	16	0.54 ²
Venlafaxine	0.00	0.00	/	0.12±0.05	0.09	25	0.14±0.06	0.12	16	<0.1 ²

¹ (Radjenović et al., 2009) for atenolol the lowest value was in MBR sludge; ² (Hörsing et al., 2011) for atenolol in activated sludge; ³ (Maurer et al., 2007); ⁴ (Sathyamoorthy et al., 2013); ⁵ (Göbel et al., 2005); ⁶ (Abegglen et al., 2009) in MBR; ⁷ (Xue et al., 2010) in conventional activated sludge and MBR sludge; ⁸ (Fernandez-Fontaina et al., 2012).

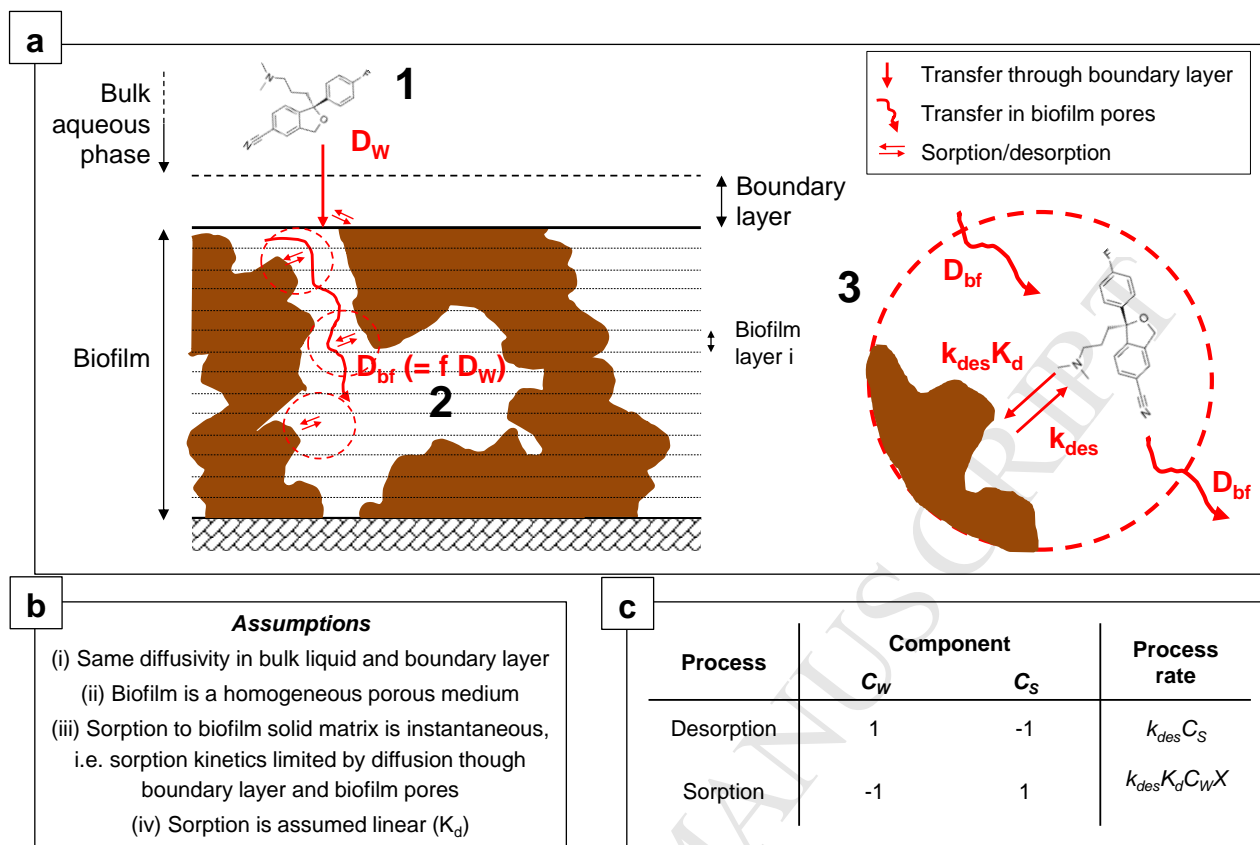


Figure 1. Conceptual model for diffusion and sorption of micropollutants into biofilms, including (a) a graphical description of the biofilm as porous medium, with discretization in 20 finite completely mixed layers, and of the consecutive steps required for partitioning onto biofilm solids (processes 1–3, see text); (b) the assumptions considered in the model; and (c) the process matrix describing sorption and desorption kinetics.

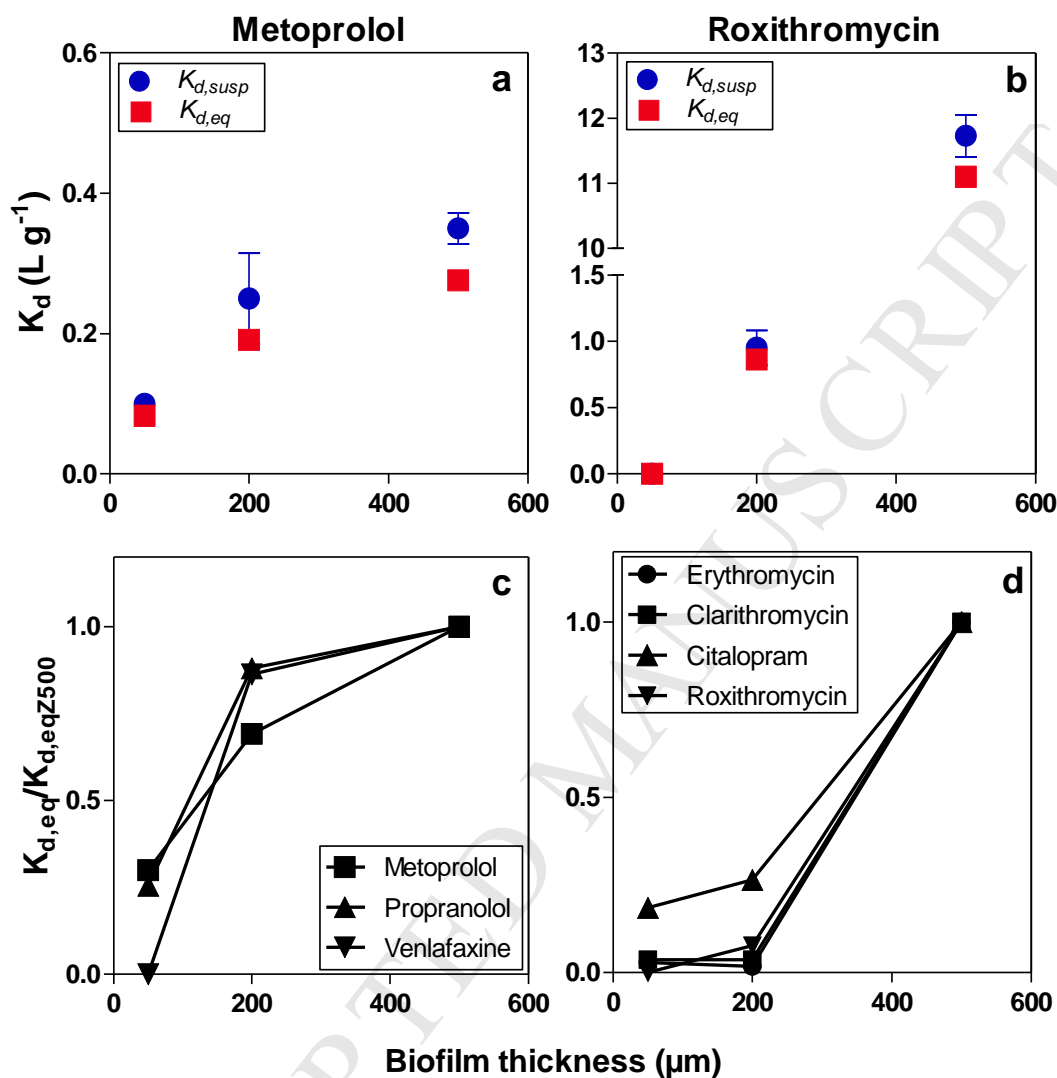


Figure 2. Values of the sorption coefficient calculated by accounting for and by neglecting biofilm porosity, $K_{d,susp}$ and $K_{d,eq}$, respectively for metoprolol (a) and roxithromycin (b). Different profiles of $K_{d,eq}$ normalized to $K_{d,eqZ500}$ (i.e., for biofilm Z500) as a function of biofilm thickness are also shown for the sorptive micropollutants (c and d).

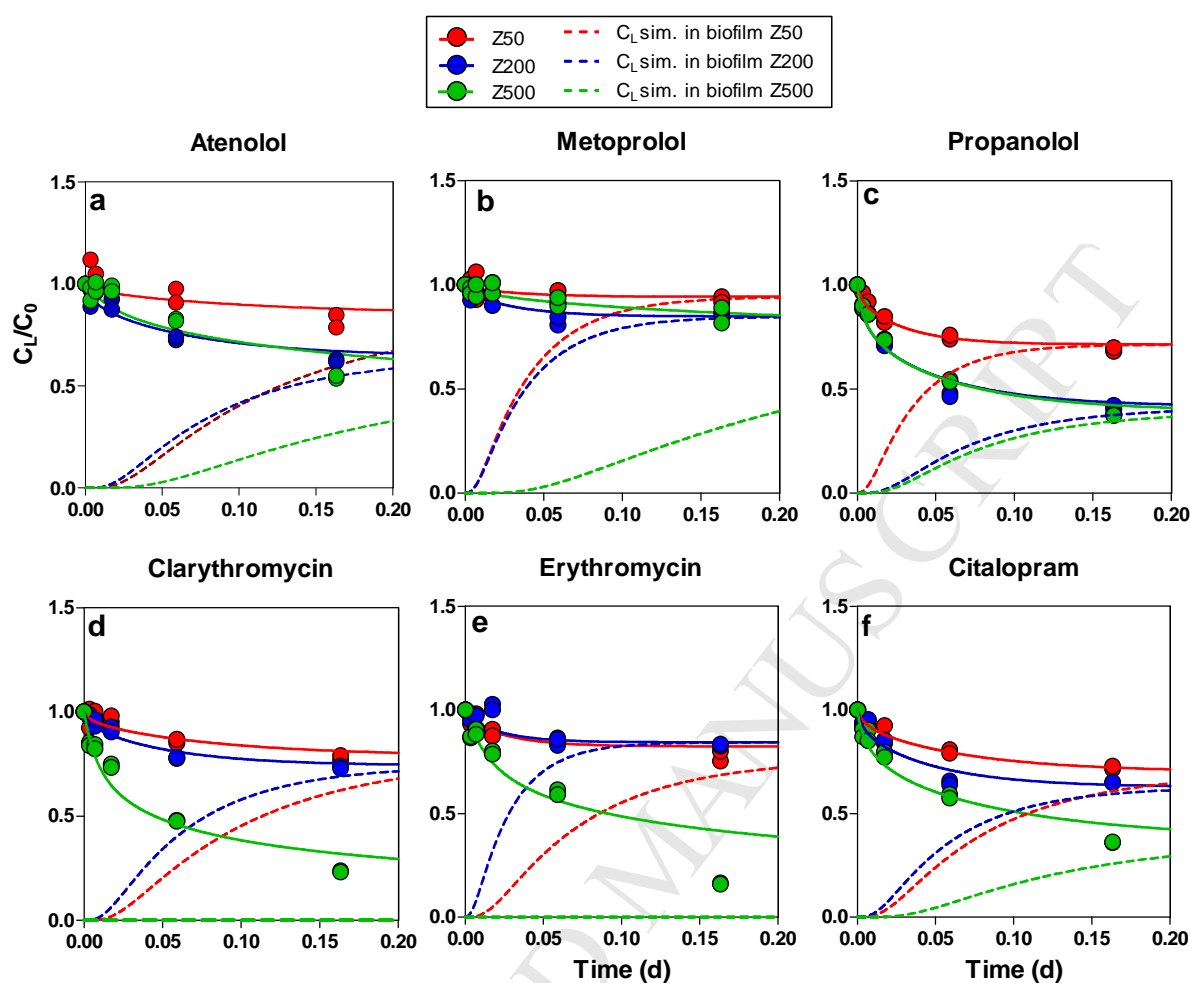


Figure 3. Measured (technical replicates, in circles) and simulated (continuous line) aqueous concentrations C_L in bulk aqueous phase (normalized over initial aqueous concentration $C_{L,0}$) and simulated concentrations in biofilm pores liquid (dashed lines) of six selected chemicals compounds during batch experiments with Z50 (red), Z200 (blue) and Z500 (green) biofilms. Simulated C_L in biofilm denotes the aqueous concentration in the deepest layer of the discretized biofilm (section 2.1).

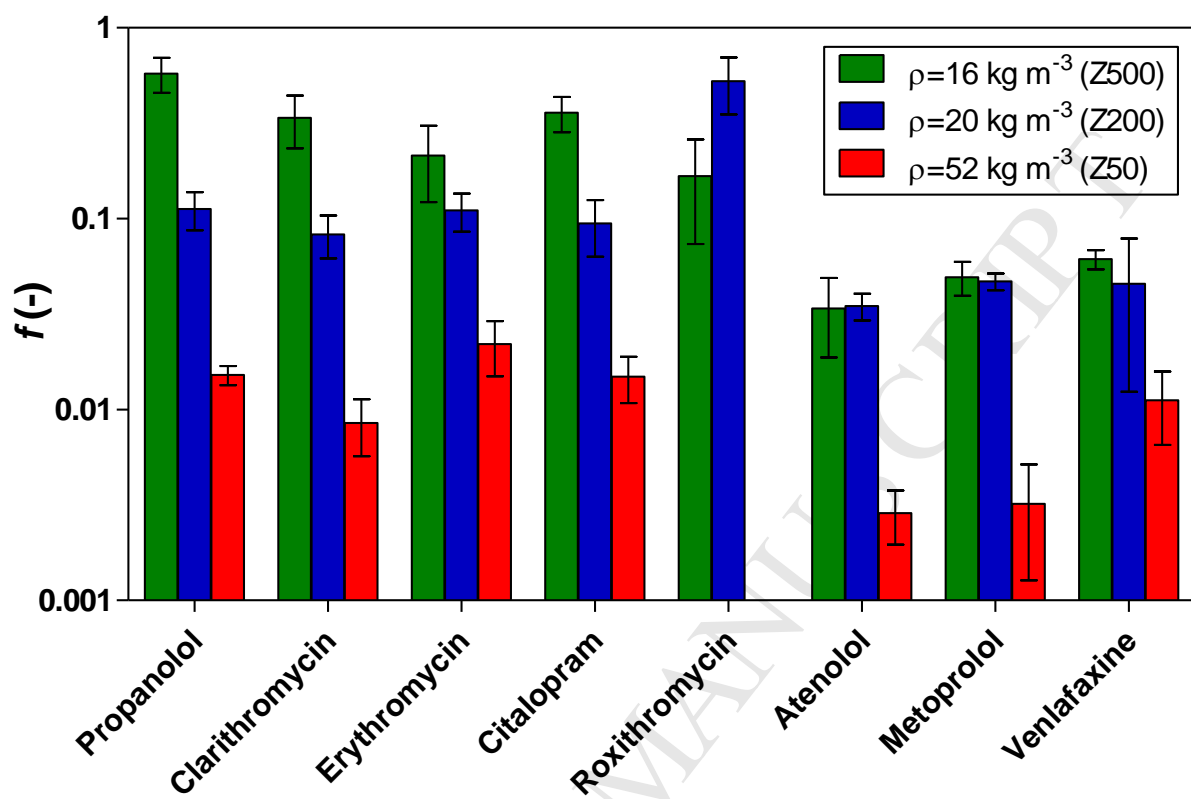


Figure 4. Estimated values of dimensionless effective diffusivity f for the three biofilm thicknesses and the eight chemicals – showing significant sorption – by calibrating the diffusion-sorption model.

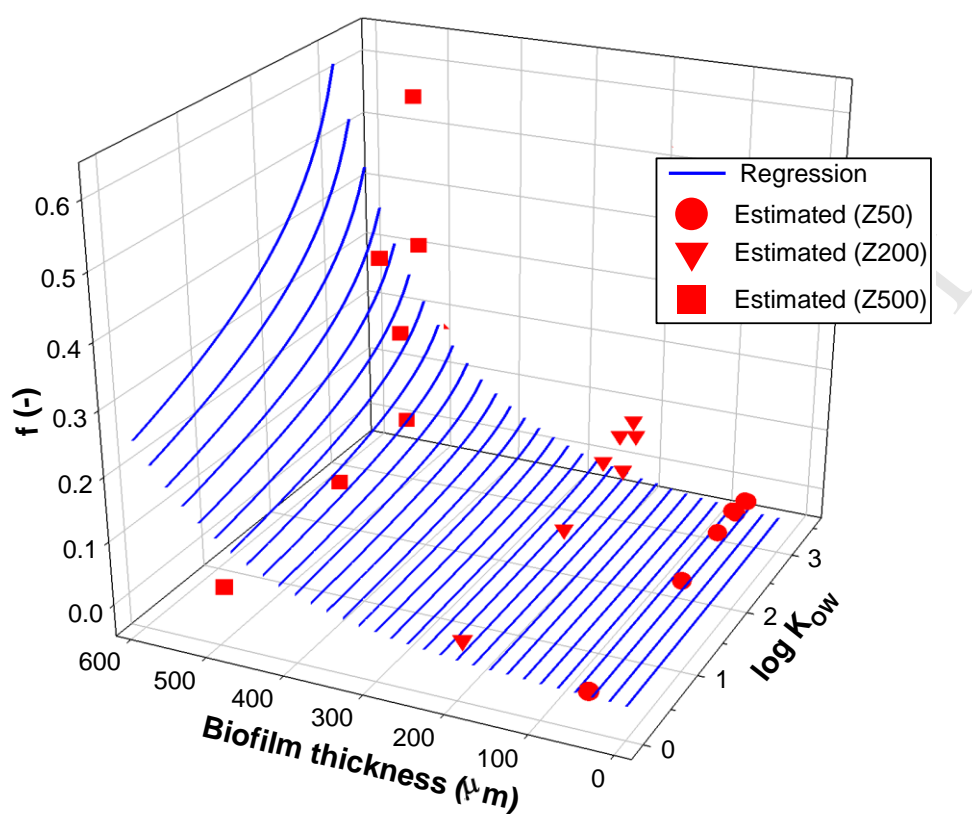


Figure 5. Plots of the empirical equation describing f – for atenolol, erythromycin, metoprolol, propranolol, clarithromycin, roxithromycin, citalopram, venlafaxine – as a function of biofilm thickness and $\log K_{ow}$, together with estimated f values (red symbols) in Z50, Z200 and Z500.

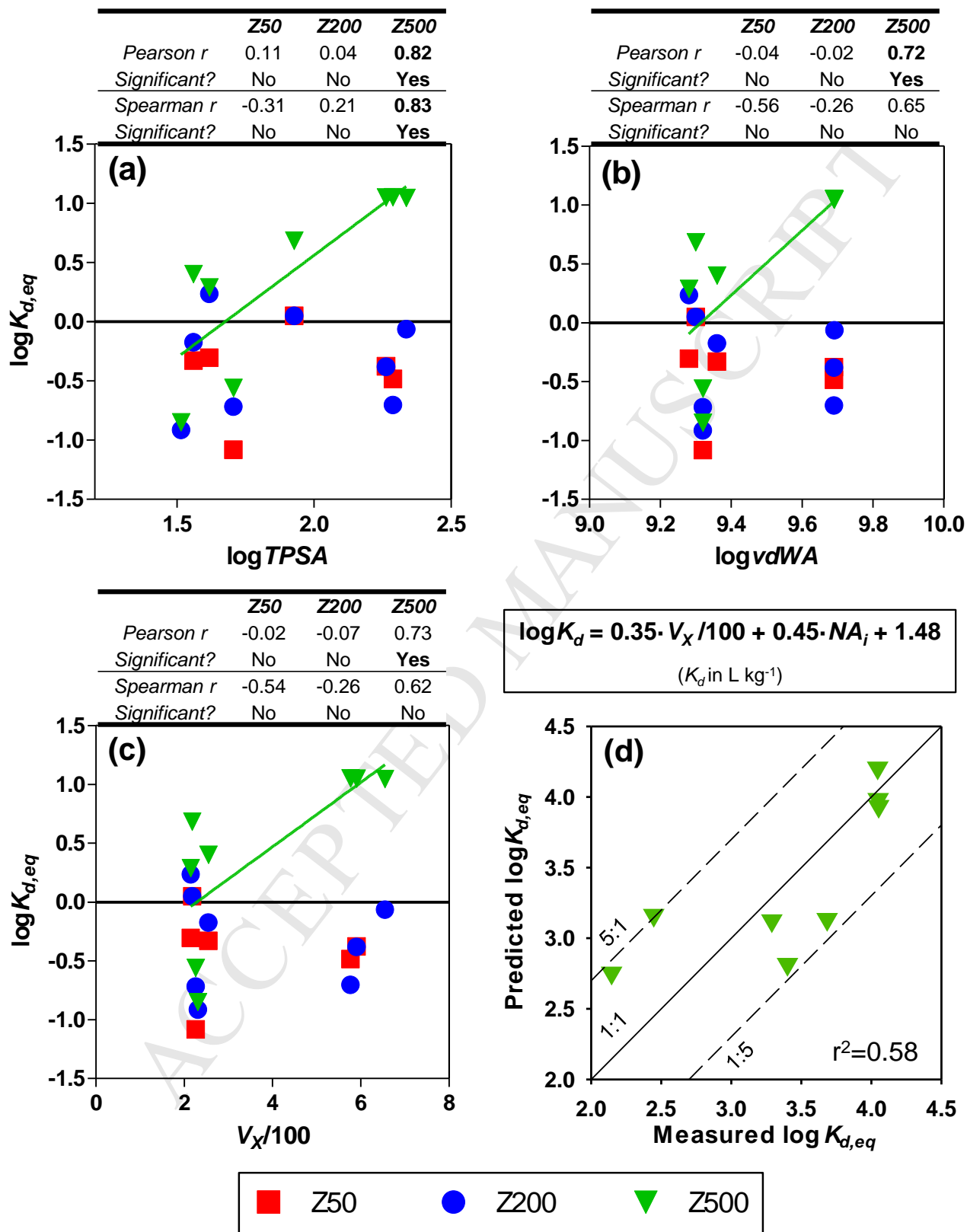


Figure 6. Correlation analysis between $\log K_{d,eq}$ of the targeted micropollutants for the three biofilms (Z50, Z200, Z500) and physico-chemical descriptors: (a) $\log TPSA$; (b) $\log vdWA$; (c) McGowan's volume V_X (divided by a factor of 100). Linear regression lines were reported only for significant correlations. Based on the correlation with $V_X/100$, an empirical regression (Eq. 8) was tested according to Droge and Goss (2013a,c). The comparison between measurements and predictions using Eq. 8 (in both cases, with $K_{d,eq}$ in $L\ kg^{-1}$) is presented in (d).

Highlights

- Diffusion-sorption of pharmaceuticals assessed in biofilms of different thicknesses
- Sorption significant only for eight positively ionized compounds
- Sorption coefficients increased with increasing biofilm thickness
- Several days necessary to reach partitioning equilibrium in thicker biofilms
- Effective diffusivity in biofilm negatively influenced by biofilm density

Supplementary Information for:

Diffusion and sorption of organic micropollutants in biofilms with varying thicknesses

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Supplementary Tables

Table S1. Diffusivity coefficients in water (D_W) of the substances exhibiting sorption, estimated according to different methods from literature (see equations below).

	Hayduk and Laudie (1974)	Wilke and Chang (1955)	$D_{W,i} (m^2 d^{-1})$		Trapp and Matthies (1998)	Sitaraman et al. (1963)
			Schwarzenbach et al. (2003)			
			I	II		
Atenolol	$4.57 \cdot 10^{-5}$	$4.82 \cdot 10^{-5}$	$4.39 \cdot 10^{-5}$	$4.10 \cdot 10^{-5}$	$7.63 \cdot 10^{-5}$	$2.72 \cdot 10^{-5}$
Erythromycin	$2.62 \cdot 10^{-5}$	$2.74 \cdot 10^{-5}$	$2.52 \cdot 10^{-5}$	$2.10 \cdot 10^{-5}$	$4.59 \cdot 10^{-5}$	$1.75 \cdot 10^{-5}$
Metoprolol	$4.33 \cdot 10^{-5}$	$4.57 \cdot 10^{-5}$	$4.16 \cdot 10^{-5}$	$3.85 \cdot 10^{-5}$	$7.61 \cdot 10^{-5}$	$2.61 \cdot 10^{-5}$
Propranolol	$4.56 \cdot 10^{-5}$	$4.81 \cdot 10^{-5}$	$4.38 \cdot 10^{-5}$	$4.09 \cdot 10^{-5}$	$7.73 \cdot 10^{-5}$	$2.72 \cdot 10^{-5}$
Clarithromycin	$2.56 \cdot 10^{-5}$	$2.67 \cdot 10^{-5}$	$2.46 \cdot 10^{-5}$	$2.04 \cdot 10^{-5}$	$4.55 \cdot 10^{-5}$	$1.72 \cdot 10^{-5}$
Citalopram	$4.20 \cdot 10^{-5}$	$4.43 \cdot 10^{-5}$	$4.04 \cdot 10^{-5}$	$3.71 \cdot 10^{-5}$	$6.91 \cdot 10^{-5}$	$2.55 \cdot 10^{-5}$
Venlafaxine	$4.31 \cdot 10^{-5}$	$4.54 \cdot 10^{-5}$	$4.13 \cdot 10^{-5}$	$3.82 \cdot 10^{-5}$	$7.47 \cdot 10^{-5}$	$2.59 \cdot 10^{-5}$
Roxithromycin	$2.48 \cdot 10^{-5}$	$2.59 \cdot 10^{-5}$	$2.38 \cdot 10^{-5}$	$1.97 \cdot 10^{-5}$	$4.30 \cdot 10^{-5}$	$1.68 \cdot 10^{-5}$

Equations

Hayduk and Laudie (1974): $D_{W,MP} = 13.26 \cdot 10^{-5} / (\eta^{1.14} MV_{MP}^{0.589})$

Wilke and Chang (1955): $D_{W,MP} = 7.4 \cdot 10^{-8} x MW^{0.5} T / (\eta MV_{MP}^{0.6})$

Schwarzenbach et al. (2003) – I: $D_{W,MP} = D_{W,ref} (MV_{ref} / MV_{MP})^{0.589}$

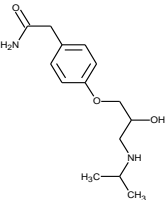
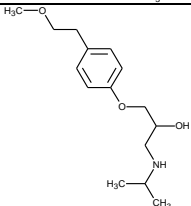
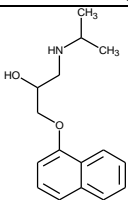
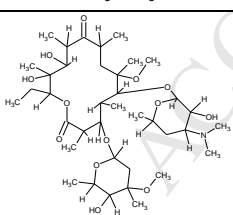
Schwarzenbach et al. (2003) – II: $D_{W,MP} = 2.3 \cdot 10^{-4} / MV_{MP}^{0.71}$

Trapp and Matthies (1998): $D_{W,MP} = D_{W,ref} (MW_{ref} / MW_{MP})^{0.5}$

Sitaraman et al. (1963): $D_{W,MP} = 5.4 \cdot 10^{-8} MW^{0.5} T L_S^{1/3} / (\eta L_S^{0.3} MV_{MP}^{0.6})$

where $D_{W,MP}$ = diffusivity of micropollutant ($=D_{W,i}$), η = viscosity of solvent/solution, MV_{MP} = molecular volume of the micropollutant, MW_{MP} = molecular weight of the micropollutant, T = temperature, $D_{W,ref}$ = diffusivity coefficient of reference substance, MV_{ref} = molecular volume of the reference substance, MW_{ref} = molecular weight of the reference substance, L_S = latent heat of vaporization of solvent at boiling point. Where required, oxygen was considered as reference substance ($D_{W,O_2} = 2.2 \cdot 10^{-4} m^2 d^{-1}$; Torresi et al., 2016). For measurement units of the different parameters, the reader is referred to the original publications.

Table S2. Physico-chemical properties of the micropollutants investigated in this study and for which sorption to MBBR biofilms was observed. Properties were estimated with ACD/Labs except for V_X (calculated according to Abraham and McGowan, 1987).

Compound	Formula	Structure	McGowan's V_X ($\text{cm}^3 \text{mol}^{-1}$)	Molecular volume MV ($\text{cm}^3 \text{mol}^{-1}$)	Molecular weight MW (g mol^{-1})	$\log K_{OW}$	$\log D$	pK_a	Ref
<i>Atenolol</i>	$\text{C}_{14}\text{H}_{22}\text{N}_2\text{O}_3$		217.6	236.6	266.34	0.1	-1.87	9.5 (base)	ACD
<i>Metoprolol</i>	$\text{C}_{15}\text{H}_{25}\text{NO}_3$		226.0	258.7	267.36	1.79	0.08	9.5 (base)	ACD
<i>Propranolol</i>	$\text{C}_{16}\text{H}_{21}\text{NO}_2$		214.8	237.1	259.34	3.1	0.96	9.5 (base)	ACD
<i>Clarithromycin</i>	$\text{C}_{38}\text{H}_{69}\text{NO}_{13}$		591.4	631.9	747.9	3.16	2.12	8.5 (base)	ACD

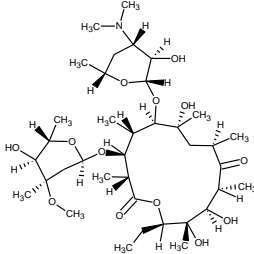
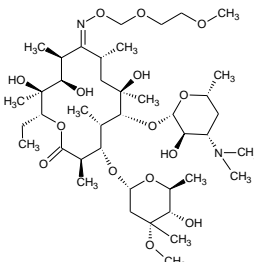
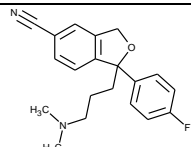
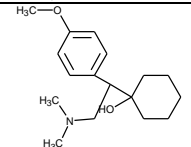
<i>Erythromycin</i>	$C_{37}H_{67}NO_{13}$		577.3	607.1	733.9	2.83	1.42	8.6 (base)	ACD
<i>Roxithromycin</i>	$C_{41}H_{76}N_2O_{15}$		655.4	666.3	837.05	3.73	2.24	8.6 (base)	ACD
<i>Citalopram</i>	$C_{20}H_{21}FN_2O$		255.3	272.6	324.39	2.51	2.06	9.4 (base)	ACD
<i>Venlafaxine</i>	$C_{17}H_{27}NO_2$		237.5	261.6	277.4	2.91	2.59	8.4 (base)	ACD

Table S3. Ionization properties and prevailing ionization state at experimental pH (=7.5) of all the micropollutants investigated in this study. Reported pK_a values are the ones relevant to typical wastewater pH. The chemicals, for which sorption to biofilms was observed, are presented in italics.

Compound	pK_a	Charge and ionic fraction at pH=7.5	Reference
Acetyl-sulfadiazine	6.99 (acid) 2.01 (base)	76% negative, 0% positive	ChemAxon
Sulfadiazine	6.5 (acid) 2.1 (base)	91% negative, 0% positive	ACD
Sulfamethizole	5.3 (acid) 1.8 (base)	99% negative, 0% positive	ACD
Sulfamethoxazole	5.7 (acid) 1.8 (base)	98% negative, 0% positive	ACD
Trimethoprim	7.0 (base)	24% positive	ACD
<i>Atenolol</i>	9.5 (base)	99% positive	ACD
<i>Metoprolol</i>	9.5 (base)	99% positive	ACD
<i>Propranolol</i>	9.5 (base)	99% positive	ACD
Sotalol	8.3 (base) 10.1 (acid)	86% positive 13% zwitterionic	ACD
<i>Clarithromycin</i>	8.5 (base)	92% positive	ACD
<i>Erythromycin</i>	8.6 (base)	92% positive	ACD
<i>Roxithromycin</i>	8.6 (base)	92% positive	ACD
Diclofenac	4.0 (acid)	100% negative	ACD
Phenazone	1.8 (base)	0% positive	ACD
Carbamazepine	Neutral		ACD
<i>Citalopram</i>	9.4 (base)	99% positive	ACD
<i>Venlafaxine</i>	8.4 (base)	90% positive	ACD
Diatrizoic acid	1.4 (acid)	100% negative	ACD
Iohexol	11.8 (acid)	0% negative	ACD
Iomeprol	11.8 (acid)	0% negative	ACD
Iopamidol	10.8 (acid)	0% negative	ACD
Iopromide	10.6 (acid)	0% negative	ACD

Table S4. Comparison between $K_{d,susp}$ (Eq. 6) and $K_{d,eq}$ (Eq. 3), calculated using the estimated asymptotic equilibrium concentration $C_{L,eq}$. The relative deviation Δ (%) between $K_{d,susp}$ and $K_{d,eq}$ is used to assess the impact of porosity on sorption coefficient estimation, i.e. the overestimation of the sorption coefficient by neglecting transport of micropollutants from bulk aqueous phase to biofilm pores.

	<i>Z50</i>			<i>Z200</i>			<i>Z500</i>		
	$K_{d,susp}$ (L g ⁻¹)	$K_{d,eq}$ (L g ⁻¹)	Δ (%)	$K_{d,susp}$ (L g ⁻¹)	$K_{d,eq}$ (L g ⁻¹)	Δ (%)	$K_{d,susp}$ (L g ⁻¹)	$K_{d,eq}$ (L g ⁻¹)	Δ (%)
Atenolol	1.15	1.12	3	1.21	1.12	8	5.15	4.84	6
Metoprolol	0.10	0.08	16	0.25	0.19	22	0.35	0.28	21
Propranolol	0.52	0.50	4	1.83	1.71	6	2.11	1.95	8
Clarithromycin	0.44	0.42	5	0.48	0.41	13	11.82	11.19	5
Erythromycin	0.35	0.33	6	0.25	0.20	22	11.91	11.28	5
Roxithromycin	0.00	0.00	/	0.95	0.86	9	11.73	11.10	5
Citalopram	0.49	0.47	5	0.74	0.67	10	2.71	2.52	7
Venlafaxine	0.03	0.00	100	0.17	0.12	30	0.21	0.14	33

Table S5. Goodness of fit (R^2) for the sorption-diffusion biofilm model, calculated by comparing measured and simulated data

	R^2		
	Z50	Z200	Z500
Atenolol	0.85	0.94	0.85
Metoprolol	0.96	0.83	0.93
Propranolol	0.99	0.97	0.98
Clarithromycin	0.93	0.97	0.94
Erythromycin	0.95	0.99	0.88
Citalopram	0.99	0.94	0.97
Venlafaxine	/	0.89	0.98
Roxithromycin	/	0.94	0.88

Table S6. Proposed models for biofilm diffusivities used in estimates validation

Diffusion model	Relation	Additional info	Range
Zhang and Bishop (1994)	$f = \varepsilon^3$	$\varepsilon = 1 - \frac{X_b}{\rho_{CELL}}$	58% < ε < 92%
Hinson and Kocher (1996)	$f = \frac{2 \cdot (1 - \varepsilon_o) \cdot \varepsilon_w}{(2 + \varepsilon_o) \cdot \left(\varepsilon_w + \frac{\varepsilon_p}{D_{pr}} \right)}$	$X_b = \varepsilon_o \cdot \rho_o + \varepsilon_p \cdot \rho_p$	
Beyenal et al. (1997)	$f = 10^{-0.0072367X_b}$		
Horn and Morgenroth (2006)	$f = 1.112 - 0.019 \cdot X_b$		10 < X_b < 20

Where f is the dimensionless diffusivity within biofilms, X_b (kg m^{-3}) is the biofilm density, ε is the biofilm porosity, ρ_{CELL} is the cells density ($250 \text{ kg} \cdot \text{m}^{-3}$ (Zhang and Bishop, 1994a)), ε_o is cells volume fraction, ρ_o is the cells density, ε_p is the extra polymeric substances (EPS) volume fraction, ρ_p is the EPS density (considered equal to ρ_o (Hinson and Kocher, 1996)), ε_w is the water volume fraction, and D_{pr} is the relative diffusivity within EPS (0.022 (Hinson and Kocher, 1996)).

Supplementary Figures

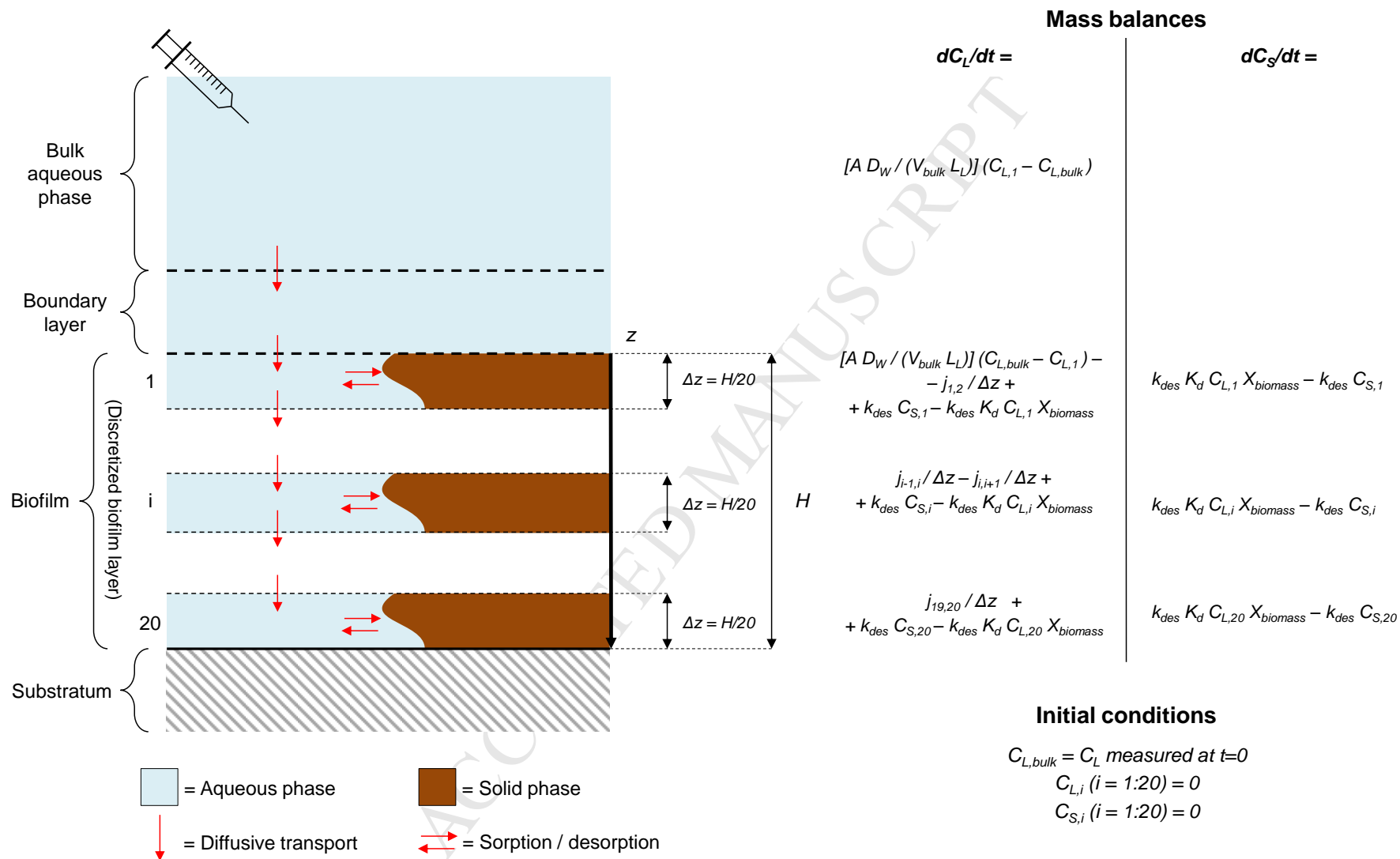


Figure S1. Conceptual representation of the biofilm model implemented in Aquasim, with mass balances for bulk aqueous phase and selected biofilm layers and initial conditions for the state variables C_L and C_S in the different compartments.

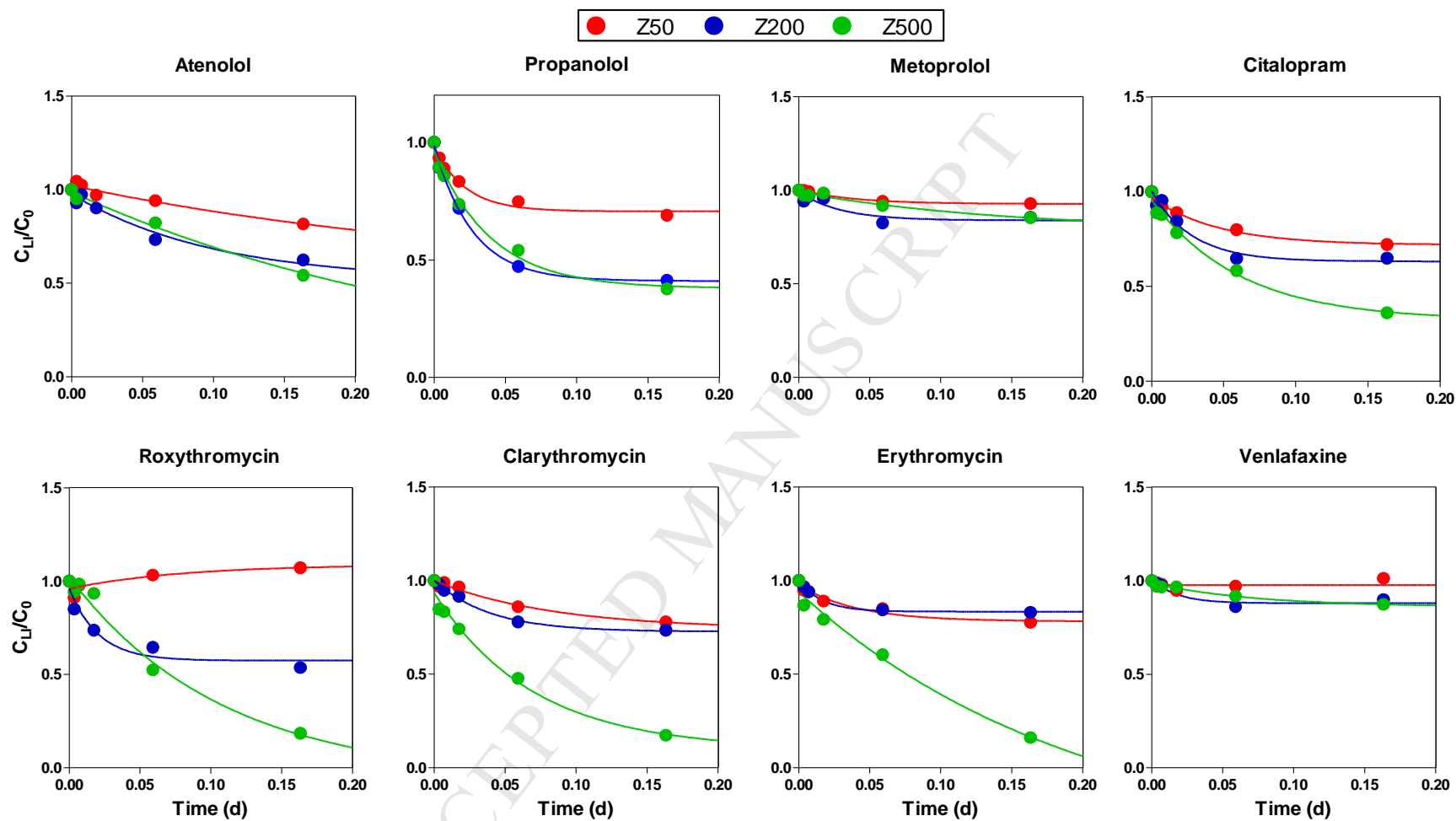


Figure S2. Model fit (continuous lines) of experimental data (circles) using first-order kinetics asymptotic function (Eq. 4) for the calculation of $K_{d,eq}$.

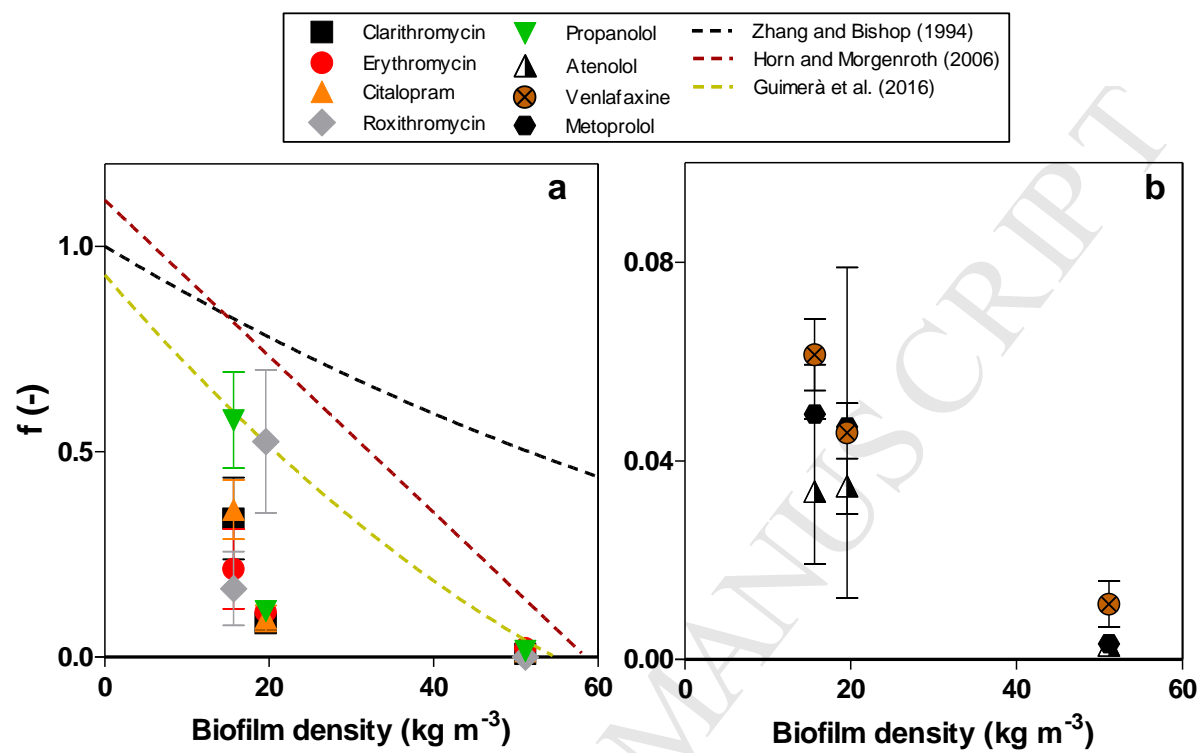


Figure S3. Estimated values of effective diffusivity coefficient f for the targeted micropollutants and regression correlation between f and biofilm density reported in literature.

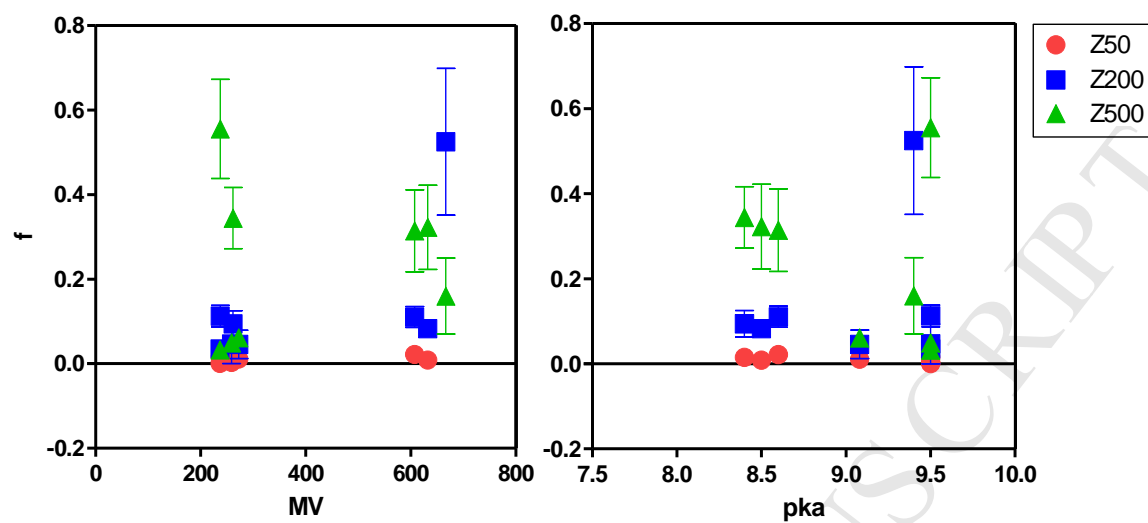
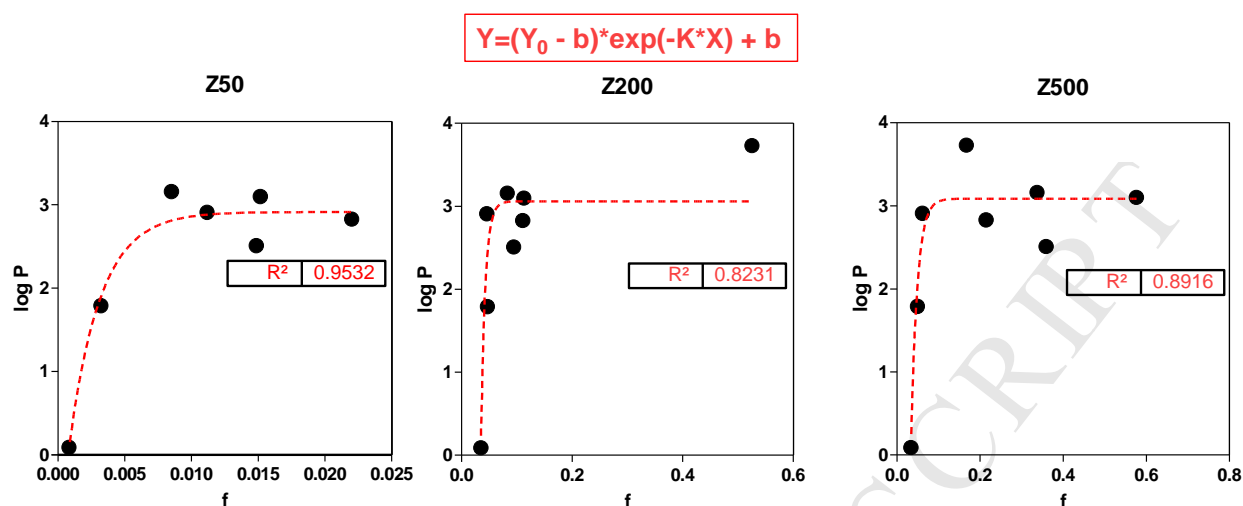


Figure S4. Estimated effective diffusivity factor (f) plotted as a function of molecular volume (MV) and dissociation constant (pK_a) of the compounds, exhibiting sorption to biofilm.



	Y0	SE Y0	b	SE b	K	SE K
Z50	-1.13	0.67	2.92	0.14	430.40	134.60
Z200	-249.40	526.10	3.06	0.25	127.50	29.79
Z500	-26.56	18.49	3.09	0.20	67.43	58.93

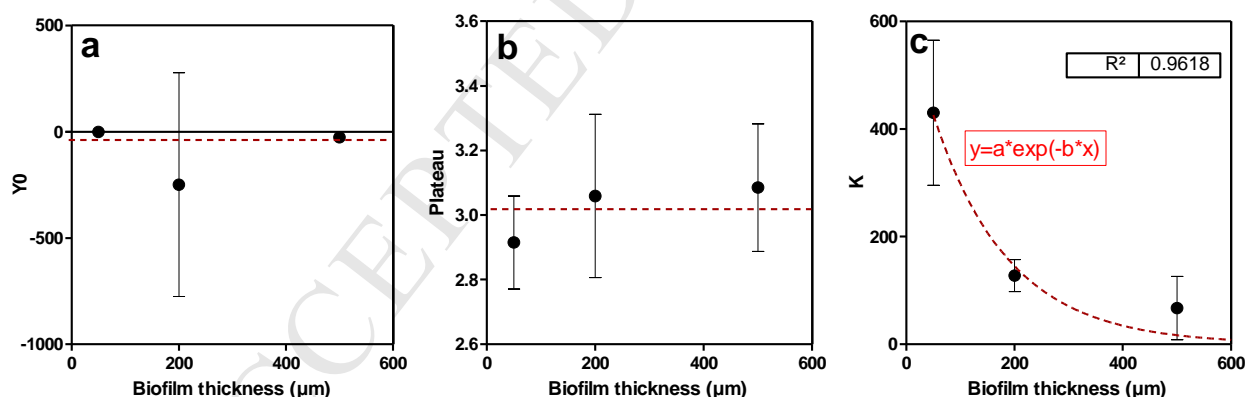


Figure S5. Summary of the regression, describing the diffusivity reduction factor f as a function of $\log K_{OW}$ ($\log P$) of the chemical and biofilm thickness. Error bars indicate standard errors of the estimated regression coefficients.

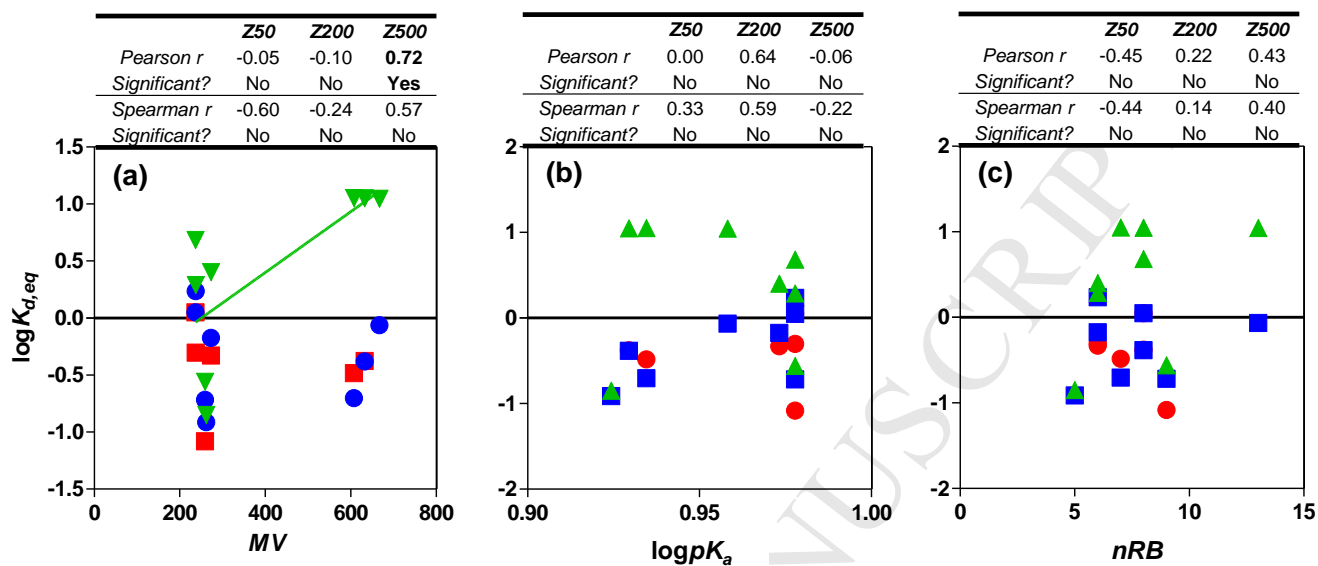


Figure S6. Partitioning coefficients ($\log K_{d,eq}$) plotted as a function of the molecular volume (MV), basic dissociation constants ($\log pK_a$) and the number of rotatable bonds (nRB).

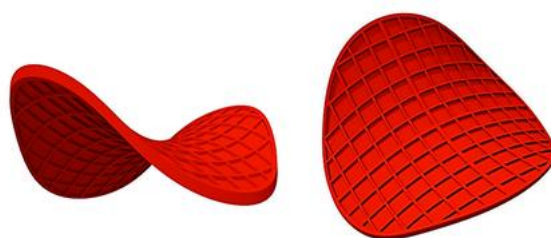


Figure S7. Drawing of Z-carriers used in this study.

Supplementary Sections

S1. Biofilm model description

The model implemented in Aquasim and used in this study is a one-dimensional biofilm model including (i) bulk aqueous phase, (ii) unstirred boundary layer and (iii) biofilm of defined maximum thickness (H), growing on an impermeable solid substratum of surface area A . The biofilm compartment consists of pore water and solid biomass, where the fraction of these two phases over the total wet biofilm volume is defined by the porosity ε and by $1-\varepsilon$, respectively. One-dimensional spatial resolution of the biofilm results in concentrations and density (hence porosity) gradients along one direction only, i.e. over the biofilm depth. The following assumptions were made as to the physical structure of the biofilm, namely (i) the biofilm is at its maximum thickness H during sorption experiments, which is equal to the 50, 200 or 500 μm depending on the type of Z-carrier; and (ii) the biofilm has constant porosity over its depth, determining constant effective diffusivity of the pharmaceutical in the biofilm. During sorption experiments (from spiking of pharmaceuticals in bulk phase until sorption equilibrium), dynamic conditions are established in the biofilm. Overall, these conditions/assumptions define a one-dimensional dynamic biofilm model. Considering diffusive transport and reaction (sorption/desorption) processes as predominant in the biofilm, the generic microscopic mass balance in the biofilm compartment for the dissolved chemical C_L is defined by the following equation (Wanner et al., 2006):

$$\frac{\partial C_L}{\partial t} = D_{bf} \frac{\partial^2 C_L}{\partial z^2} + r \quad (\text{Eq. S1})$$

where the term r denotes the rates of reaction processes. The specific form of this equation, where reaction processes included sorption to and desorption from biofilm, had already been included in the manuscript (Eq. 2).

As to the bulk aqueous phase, a mass balance can also be established considering that (i) no reaction occurs; and (ii) transfer of spiked pharmaceuticals from bulk aqueous phase to biofilms occurs via diffusive transport through the unstirred boundary layer. Under the assumptions of constant bulk aqueous phase volume (V_{bulk}) and no reaction occurring in bulk phase, the mass balance for the dissolved concentration is defined as (Wanner et al., 2006):

$$V_{bulk} \frac{dC_L}{dt} = \frac{AD_W}{L_L} (C_{L,bf} - C_{L,bulk}) \quad (\text{Eq. S2})$$

where $C_{L,bulk}$ and $C_{L,bf}$ are the dissolved concentrations in bulk phase and at biofilm surface, respectively, A is the bulk-biofilm exchange area and (equivalent to the area covered by biofilm), D_W the diffusivity in free water and L_L the boundary layer thickness. Given that $C_{L,bf} < C_{L,bulk}$ at $t=0$, an outward flux of dissolved pharmaceutical from bulk phase to the biofilm is established.

In this form, the model is a combination of ordinary and partial differential equations. The latter can be solved using the method lines, with discretization of the biofilm compartment into n layers (in this study $n=20$), each having the same thickness $\Delta z = H/n$. Layer 1 denotes the top biofilm, while layer 20 denotes the deepest part of the biofilm. This allows for a numerical approximation of the spatial derivative, thus for a simplification of the mathematical model from one-dimensional (with one set of partial differential equations) to zero-dimensional (with n sets of ordinary differential equations, one set for each layer). The mass balance is established in each layer for the two state variables C_L and C_S , where the latter is assumed to undergo negligible diffusive transport within the solid matrix (i.e., cannot be transported upwards or downwards in the biofilm). As described above, the mass balance for C_L included sorption, desorption and downward diffusion in each of the biofilm layers, not only in the deepest layer. Given the conditions established in the experiment and its short duration, advective transport of solubles and biofilm detachment were neglected.

The microscopic mass balance in a generic layer (i) for the dissolved mass of pharmaceuticals, considering predominant downward diffusive transport, is written as:

$$dm_{L,i} / dt = \text{Diffusive mass transfer from layer } (i-1) \text{ (or bulk phase)} - \text{Diffusive mass transfer to layer } (i+1) + \text{Desorption from solids in layer } i - \text{Sorption to solids in Layer } i$$

Diffusive mass transfer from the upper adjacent layer and to the adjacent lower layer (occurring only in pore water phase) defines the connection between different layers of the biofilm, and the “driving force” is given by the difference in dissolved concentration between adjacent layers. Mass balances in different compartments and layers, relevant to the experiments presented in this study, are given below:

- Bulk aqueous phase

$$C_L: \frac{dC_{L,bulk}}{dt} = \frac{AD_W}{L_L V_{bulk}} (C_{L,1} - C_{L,bulk}) \quad (\text{Eq. S3})$$

C_S : no sorbed pharmaceuticals are present in bulk phase in the absence of suspended solids

- Layer 1 (top biofilm) – C_L and C_S in layer 1 are denoted with the subscript ‘1’

$$C_L: \frac{dC_{L,1}}{dt} = \frac{AD_W}{L_L V_{bulk}} (C_{L,bulk} - C_{L,1}) - \frac{j_{1,2}}{\Delta z} - k_{des} K_d C_{L,1} X + k_{des} C_{S,1} \quad (\text{Eq. S4})$$

$$C_S: \frac{dC_{S,1}}{dt} = k_{des} K_d C_{L,1} X - k_{des} C_{S,1} \quad (\text{Eq. S5})$$

- Layer i (inner biofilm) – C_L and C_S in layer i are denoted with the subscript ‘ i ’. Layers $(i - 1)$ and $(i + 1)$ denote the layer above and below layer i .

$$C_L: \frac{dC_{L,i}}{dt} = \frac{j_{i-1,i}}{\Delta z} - \frac{j_{i,i+1}}{\Delta z} - k_{des} K_d C_{L,i} X + k_{des} C_{S,i} \quad (\text{Eq. S6})$$

$$C_S: \frac{dC_{S,i}}{dt} = k_{des} K_d C_{L,i} X - k_{des} C_{S,i} \quad (\text{Eq. S7})$$

- Layer 20 (deep biofilm) – C_L and C_S in layer 20 are denoted with the subscript ‘20’

$$C_L: \frac{dC_{L,20}}{dt} = \frac{j_{19,20}}{\Delta z} - k_{des} K_d C_{L,20} X + k_{des} C_{S,20} \quad (\text{Eq. S8})$$

$$C_S: \frac{dC_{S,20}}{dt} = k_{des} K_d C_{L,20} X - k_{des} C_{S,20} \quad (\text{Eq. S9})$$

The generic term $j / \Delta z$ ($\text{g m}^{-3} \text{ d}^{-1}$) describes the diffusive mass transfer between adjacent layers as a simplification of the second order derivative $D_{bf} \partial^2 C / \Delta z^2$. The two subscripts of the mass flux j ($\text{g m}^{-2} \text{ d}^{-1}$) indicate the layer from which and to diffusive mass transfer occurs, respectively. Based on Fick’s first law of diffusion ($j = -D \partial C / \partial z$), the flux j is a function of the effective diffusivity D_{bf} and of the concentration gradient between adjacent layers, i.e. the driving force for diffusive transport. Further

details on the numerical methods used to approximate partial differential equations to ordinary differential equations through spatial discretization can be found in Reichert (1994).

Initial conditions for C_L and C_S in different compartments were established based on measurements and assumptions, i.e.:

Bulk aqueous phase

$$C_L = C_L \text{ measured at } t=0$$

$$C_S = 0 \text{ (no solids were assumed to be present in the bulk aqueous phase, since the effluent was pre-filtered – see Lines 225–226)}$$

Biofilm (both pore water and solids in each layer i)

$$C_{L,i} = 0 \text{ (the pore water at } t=0 \text{ did not contain any mass of pharmaceuticals, since spiking occurred in the bulk aqueous phase)}$$

$$C_{S,i} = 0 \text{ (the biofilm was pre-washed with tap water overnight to allow for desorption of previously sorbed pharmaceuticals – see also Lines 223–224)}$$

A schematic representation of the system and of the biofilm discretization, as well as of the mass balances in the system and initial conditions for the state variables, is shown in Figure S1.

S2. Biofilm properties

Biomass dry density in biofilm (ρ_d , g cm⁻³) was calculated based on Eq. S10 (Tchobanoglous and Burton 1991; Hu et al., 2013):

$$\frac{M_s}{\rho_d} = \frac{M_f}{\rho_f} + \frac{M_v}{\rho_v} \quad (\text{Eq. S10})$$

where M_s (g) is the dry mass of biofilm solids (expressed as total attached solids, TAS), M_f (g) the dry mass of fixed mineral solids in the biofilm (expressed as total fixed solids, TFS), ρ_f the density of fixed solids (=2.5 gTFS cm⁻³), M_v (g) the dry mass of volatile solids in the biofilm (expressed as total volatile solids, TVS), and ρ_v the density of volatile solids (=1 gTVS cm⁻³).

The biofilm volume not occupied by pores, i.e. including water inside the cells but excluding water outside the cells, was calculated as (Eq. S11):

$$V_w = \frac{\frac{M_s}{\rho_d}}{1 - W_{wi}} \quad (\text{Eq. S11})$$

where W_{wi} is the water content inside the cells (=80% of total cell biomass) (Hu et al., 2013; Zhang and Bishop, 1994b). Thus, biofilm porosity (%) was calculated as (Eq. S12):

$$\varepsilon = 1 - \frac{V_w}{V_{bf}} \quad (\text{Eq. S12})$$

where V_{bf} (m^3) is the total biofilm volume (volume of wet biofilm including pore water volume, determined from nominal surface area and biofilm thickness of each Z-carrier type). The pore water volume V_{PW} (m^3) was eventually determined as (Eq. S13):

$$V_{PW} = \varepsilon \cdot V_{bf} = V_{bf} - V_w \quad (\text{Eq. S13})$$

Finally, the biofilm density (gTAS m^{-3}) was calculated as (Eq. S14):

$$\rho = \frac{M_s}{V_{bf}} \quad (\text{Eq. S14})$$

We note that biofilm density ρ (Eq. S14) denotes the mass of (microbial) biomass per volume of wet biofilm (i.e., defines a concentration within the biofilm), while the dry biofilm density ρ_d denotes the weight of dry biofilm per volume of dry biofilm (i.e., defines a *true* density).

The thickness of the boundary layer, L_L (μm), was assumed to be equal to 10 μm for all the Z-carriers. L_L can be estimated, based on fluid dynamics principles, as a function of the characteristic length of the carrier L_C (the flow-through radius of the biofilm carrier minus the biofilm thickness) and the non-dimensional Sherwood number (Boltz et al., 2011). When considering Z-carriers design, L_C is minimized as most of the interstitial space is occupied by the biofilm (Torresi et al., 2016). Thus, L_L was selected by considering the lowest value reported in literature (Brockmann et al., 2008; Joss et al., 2004). It is likely that the high flow rate of nitrogen sparging during batch experiments may have further minimized L_L , as previously considered (Wicke et al., 2007). Furthermore, comparable L_L values have been used for fate modelling of illicit drugs (having similar $D_{w,i}$ with the chemicals assessed in this study) in sewer biofilms (Ramin et al., submitted).

S3. Derivation of the adjusted partition coefficient (Equation 5)

Based on mass conservation principles, the mass of pharmaceuticals spiked at $t=0$ in the bulk phase is equivalent to the total mass at the end of sorption experiments. Hence, the following mass balance can be written (in the absence of any biological or abiotic degradation of pharmaceuticals):

$$\text{Mass spiked in bulk phase (t=0)} = \text{Remaining mass in bulk phase} + \text{Mass dissolved in pore water} + \text{Mass sorbed to biofilm solids}$$

The above mass balance can be translated in the following equation:

$$C_{L,0} V_{bulk} = C_{L,eq} V_{bulk} + C_{L,eq} V_{PW} + C_{S,eq}^* M_{X,biomass} \quad (\text{Eq. S15})$$

where $C_{L,0}$ and $C_{L,eq}$ [$\mu\text{g L}^{-1}$] are the dissolved pharmaceuticals concentrations at $t=0$ and at equilibrium, respectively; $C_{S,eq}^*$ [$\mu\text{g g}^{-1}$] is the sorbed concentration and the superscript ‘*’ is used to distinguish it from $C_{S,eq}$ [$\mu\text{g L}^{-1}$] as defined in the main text; V_{bulk} and V_{PW} [L] are the volumes of bulk aqueous phase and pore water, respectively; and $M_{X,biomass}$ [g] is the mass of solids in the system.

The mass of solids can be defined as the product of the concentration of solids in the system and the total volume of the system:

$$M_{X,biomass} = X_{biomass} (V_{bulk} + V_{bf,wet}) \quad (\text{Eq. S16})$$

where $X_{biomass}$ [g L^{-1}] denotes the concentration of solids as defined in the manuscript text (0.8 g L^{-1}) and $V_{bf,wet}$ [L] is the volume of wet biofilm (= surface area of Z carriers · biofilm thickness, also equal to the sum of pore water volume and volume occupied by solids in biofilm).

Hence, the last term of the sum can be rearranged as:

$$C_{S,eq}^* M_{X,biomass} = C_{S,eq}^* X_{biomass} (V_{bulk} + V_{bf,wet}) = C_{S,eq} (V_{bulk} + V_{bf,wet}) \quad (\text{Eq. S17})$$

where $C_{S,eq}$ [$\mu\text{g L}^{-1}$] is the sorbed concentration of pharmaceuticals as defined in the main text (e.g., Eqs. 3 and 4).

By rearranging the mass balance, we can write:

$$C_{S,eq} (V_{bulk} + V_{bf,wet}) = C_{L,0} V_{bulk} - C_{L,eq} V_{bulk} - C_{L,eq} V_{PW} \quad (\text{Eq. S18})$$

and it follows that:

$$C_{S,eq} = C_{L,0} V_{bulk} / (V_{bulk} + V_{bf,wet}) - C_{L,eq} (V_{bulk} + V_{PW}) / (V_{bulk} + V_{bf,wet}) \quad (\text{Eq. S19})$$

Eventually, the adjusted partition coefficient K_d (accounting for the pharmaceutical concentration dissolved in pore water) can be written as:

$$K_d = C_{S,eq} / (C_{L,eq} X_{biomass}) = [C_{L,0} V_{bulk} / (V_{bulk} + V_{bf,wet}) - C_{L,eq} (V_{bulk} + V_{PW}) / (V_{bulk} + V_{bf,wet})] / (C_{L,eq} X_{biomass}) \quad (\text{Eq. S20})$$

which is also presented as Equation 5 in the main manuscript text.

S4. Proposed empirical correlation for effective diffusivity coefficient f

An exponential equation was first used to correlate f with $\log K_{ow}$ at each biofilm thickness (Fig. S3). Secondly, the intercept (y_0), the asymptotic coefficient b (corresponding to the maximum $\log K_{ow}$) and the slope (k) (see Fig. S4) estimated separately for Z50, Z200 and Z500 were plotted against biofilm thickness (Fig. S4, a–c). While no trend was observed for y_0 and b with biofilm thickness, a second exponential equation was used to correlate values of slope (k) of the three biofilms with biofilm thickness. The obtained relationship is presented in Eq. S21

$$f = \frac{1}{488 \cdot e^{-0.0072L_F}} \ln \left(\frac{-12.7 - \log K_{ow,max}}{\log K_{ow} - \log K_{ow,max}} \right) \quad (\text{Eq. S21})$$

where L_F is the biofilm thickness (μm) and $\log K_{ow,max}$ is the asymptotic $\log K_{ow}$ which approximate the one reported for the targeted compounds in this study.

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